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THE EFFECT OF THE FEMALE LIFE CYCLE ON MEASUREMENTS OF SELENIUM STATUS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the degree Doctor of Philosophy in the Graduate School of The Ohio State University

By
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ABSTRACT

Selenium status is known to be affected by various factors including age, gender, health status, and dietary intake of selenium. It also has been reported that estrogen influences selenium status. Estrogen, the major ovarian steroid hormone, has a critical impact on woman's health. To study whether the specific stage of life, during which major changes in the hormonal profile occur, affect selenium status, females at different stages of the life and reproductive cycle were investigated. Selenium concentrations and glutathione peroxidase activities in plasma/serum and red blood cells were measured during three stages of the female life cycle; early puberty, the menstrual cycle and postmenopause. Dietary selenium intake was also assessed to determine whether selenium intakes were sufficient to maintain adequate selenium status at each stage of the life cycle.

To determine selenium status and intakes prior to menarche, 40 young adolescents in the early pubertal stage were studied. To examine the relationship between fluctuations in circulating estrogen and selenium status in blood during the menstrual cycle, selenium status and estrogen levels were measured in 14 young premenopausal women at four time points during one menstrual cycle: early follicular phase (EF: day 1-3 of menses), periovulatory
phase (PO: estrogen peak preceding ovulation) and mid luteal phase (ML: 7-9 day after estrogen peak). The estrogen peak was determined by using urinary luteinizing hormone (LH) concentrations that reflect the LH surge in the blood. To study the effect of menopause on selenium status, serum estrogen and selenium parameters were measured in 10 healthy postmenopausal women and compared to those of 13 healthy premenopausal women. Blood samples from premenopausal women were collected during the EF phase when the estrogen concentration is the lowest. To minimize the variations that could come from differences in dietary intake of selenium, early screening of selenium intake by food frequency was performed.

Young females who were approaching menarche appeared to consume adequate amount of dietary selenium (double the selenium RDA), which were reflected in measurements of selenium status that were comparable to values from healthy adults. RBC selenium concentrations were correlated with body mass index and weight, the indices of rapid growth at that stage. During the menstrual cycle measurements of selenium status, except RBC selenium, showed cyclic changes similar to the fluctuation in estrogen levels. Selenium measurements were highest during the PO phase, and lowest during the EF phase. Dietary selenium intakes did not vary throughout the menstrual cycle, suggesting that the phase-related fluctuation of selenium status was independent of dietary changes. Postmenopausal women, in the third study, maintained an optimum selenium status which was comparable to that of premenopausal women's. Dietary selenium intakes of the healthy postmenopausal women was
also adequate and above the RDA. GPx activity in postmenopausal women, however, was greater than that of premenopausal women, suggesting that age-related changes in oxidative stress might increase GPx activity.

In conclusion, young females were shown to have adequate selenium intakes and status during early puberty despite the low circulating estrogen concentrations that occur during this stage of the life cycle. During the menstrual cycle, selenium measurements were within the normal range but, with the exception of RBC selenium concentration, fluctuated coinciding with cyclic changes in estrogen concentration. This fluctuation was independent of dietary selenium intakes since the selenium intakes of those females remained constant over the cycle. Postmenopausal women were shown to have adequate selenium intakes and status. GPx activity in postmenopausal women, however, was elevated compared to that of premenopausal women. Since dietary selenium intakes and estrogen concentration in premenopausal and postmenopausal women did not differ, this result suggests that other factors affect selenium status and/or antioxidant capacity after menopause.
Dedicated to my Lord, Jesus Christ,

and

my parents with love
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CHAPTER 1

INTRODUCTION

A woman's body undergoes dramatic physiological and hormonal changes throughout the life cycle. These hormonal fluctuations have been shown to affect overall health. The first hormonal changes occur during puberty and affect sexual maturation including appearance of pubic hair, breast development, and menarche. During the active reproductive years, various hormones interact with each other to regulate the menstrual cycle or to support pregnancy. In later life, as a woman approaches menopause, her body experiences physiologic and clinical changes with the diminution in circulating estrogen.

In spite of this unique female physiology, its effects on the nutritional needs for females, including the need for selenium, have been overlooked. As an essential nutrient for humans and animals, selenium is a constituent of the selenium dependent enzyme, glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide and lipid peroxides. Therefore, selenium plays
an important role as an antioxidant protecting cells against peroxide induced damages. Low selenium concentrations are associated with liver necrosis, muscular myopathy, heart diseases, and some cancers. The Recommended Dietary Allowances (RDA) for selenium include requirements for infants, children, and adults but, are based on studies done on males only (Yang et al. 1987). With the exception of body weight, no developmental factors related to age or gender were considered in the establishment of the selenium recommendation since little is known about their effects on selenium status.

Blood selenium concentrations are affected by numerous factors including geography (Luo et al. 1985), age (Bortoli et al. 1991), health status (Cser 1993), and dietary intake (Åkesson et al. 1997). Several lines of evidence suggest that fluctuations in female reproductive hormones also affect measurements of selenium status. A preliminary report has indicated that selenium status fluctuates during the menstrual cycle (McAdam 1994) and blood selenium concentrations have been shown to fluctuate during the rat estrus cycle (Smith et al. 1995). These cyclic fluctuations in selenium status, both in the woman and the rat, appear to coincide with the pattern of estrogen fluctuation. Blood selenium and glutathione peroxidase activities (GPx) have also been shown to be lower in post-menopausal women than in younger women (Campbell et al. 1989). During pregnancy, a time of unique hormonal fluctuations including that of estrogen, blood selenium concentrations have been shown to decrease, independently of selenium intake (Smith and Picciano 1986). The possible effects of estrogen on selenium status in females are important to understand
since the crucial times for women to be in optimal selenium status coincide with the occurrence of major hormonal changes (e.g., adolescence, pregnancy, and postmenopause).

Postmenopausal women experience an acceleration in the aging process and are at an enhanced risk for certain diseases, some of which are associated with low selenium status (e.g., CVD, cancer, rheumatoid arthritis) and lack of estrogen. In particular, selenium plays a role in protecting membrane lipids against peroxidation during the aging process (Reddy et al. 1981), and there have been reports that low blood selenium is related to low estrogen status and increased cardiovascular disease (CVD).

The goal of this study was to determine the relationships among measurements of selenium status, selenium intake, and changes in estrogen status during three stages of the female life cycle (early puberty, the menstrual cycle, and postmenopause) that are associated with significant hormonal changes. Experiments were designed to investigate the following questions: 1) Do young females prior to menarche maintain the adequate selenium intakes and status? 2) Do parameters of selenium status fluctuate coinciding with the physiological variation of estrogen concentration during the menstrual cycle? 3) Does menopause affect the selenium status measurements when compared to those of premenopausal women? The research hypotheses for the study were the following: 1) Early adolescent girls maintain suboptimum selenium status, which results from the possible low intake of selenium and low estrogen concentration; 2) Measurements of selenium status would change during the
menstrual cycle along with the physiological fluctuation of plasma estrogen concentration, independent of selenium intake; and 3) Selenium status of postmenopausal women would be lower than that of premenopausal women due to a decrease in selenium intake and circulating estrogen levels.

This study was designed to determine the selenium status at three points during the life cycle when the female body undergoes dramatic changes in the hormonal profile. The estrogen status at each point of the life cycle was carefully considered to detect the possible effect of estrogen on the status of selenium. The results of this study will further our understanding of the relationship between selenium and estrogen metabolism throughout the female life cycle.

The results may also provide useful information for assessment of selenium status during the menstrual cycle and for the establishment of recommendations of selenium intake for females throughout the life span. In addition, for postmenopausal women, the results of this study will help determine if supplemental selenium, with or without estrogen replacement, should be investigated in terms of prevention of diseases which are believed to be associated with oxidative damage including cardiovascular disease and cancer.
CHAPTER 2

REVIEW OF LITERATURE

General Information of selenium

Selenium is an essential element both for human and animal growth at lower concentrations and a toxic substance at higher concentrations (Shamberger 1986). It is one of the rarest elements taking 70th position in abundance among the 88 elements that naturally occur in the earth’s crust (Nebergall 1968). Selenium lies between sulfur and tellurium in group VI of the periodic table and is metalloid having both properties of metal and nonmetal. Selenium exists in various oxidation states: -2, 0, +2, +4, and +6 (Cooper et al. 1974) and elemental selenium can be oxidized to selenite (+4) or selenate (+6), or reduced to selenide (-2).

Although selenium is widely found over the surface of the earth, it is not distributed evenly over the world. Mainly depending on geochemical factors, especially the nature of the parent rocks, soil concentration of selenium occurs in greater or lesser amounts than the average range, which is between 1.0 and 1.5 micrograms/gram. Alkaline and fairly dry soils that contain sufficient amounts of
selenium to produce toxic plants are referred to as seleniferous soils. Those toxic soils are found in much of the northern Great Plains including, South Dakota, North Dakota, Colorado, Montana and Wyoming, and the Southwest including Utah, Arizona and New Mexico (Rosenfeld and Beath 1964). In contrast, some of the regions of the world that have soils with low selenium concentrations include Denmark, eastern Finland, New Zealand and a long-belt region from northeast to south central of China (Combs and Combs 1986).

The earliest written report of selenium is thought to be the description by Arnold of Villanova, in the 13th Century. Marco Polo recorded the first selenium poisoning by describing a necrotic hoof disease of horses that occurred in China in the 13th Century (Polo 1967). Later, selenium was isolated and characterized in 1817 by the Swedish chemist, Berzelius (1818) as a red deposit on the walls of a lead chamber used in the production of sulfuric acid. It, however, was not until 1957 that the biological essentiality of this element was acknowledged by Schwartz and Foltz when they presented that a selenium salt prevented liver necrosis in rats (Schwartz and Foltz 1957). Up until that time, selenium had been known to have undesirable characteristics especially having toxic effects when it exists at high levels. In the same year, several other studies suggested a preventive role of selenium on exudative diathesis in chicks, a condition caused by consuming a vitamin E deficient diet. These studies confirmed the nutritional significance of selenium as well (Schwartz et al. 1957, Patterson et al. 1957).

In 1973, several researchers brought attention to the physiological role of selenium by reporting that selenium is a functional part of the enzyme,
glutathione peroxidase (GPx), in rat erythrocytes (Rotruck et al. 1973, Flohe et al. 1973). This finding led to a broader conception that selenium played a role in the cellular antioxidant system, in which it had both an independent function as a component of GPx or a complementary function as a component of GPx and vitamin E.

In 1979 the clinical importance of selenium begun to be emphasized when it was reported that selenium supplementation prevented the endemic cardiomyopathy, Keshan disease in China (Keshan Disease Research Group 1979a). This was the first report of a disease state being related to selenium deficiency, suggesting the important role of selenium in human health. Since then, the association between selenium and disease states in humans, including cardiovascular disease (Shamberger 1978) and various cancers (Wattenberg 1978, Ip 1986), have been investigated widely. To further identify the biological role of selenium, the investigation of selenium has been accelerated, leading to the discovery of eleven selenoproteins some of which are critical in human health and growth.

**Biological Role of Selenium**

**Reactive Oxygen Species and Antioxidant System**

During normal aerobic metabolism, reactive oxygen species (ROS) are produced. They also can be generated when cells are exposed to certain forms of stress. ROS are substances that contain oxygen and have higher reactivity
than ground-state molecular oxygen. These include oxygen radicals such as superoxide, hydroxyl, and peroxyl radical, and even non-radical molecules like hydrogen peroxide and singlet oxygen. At low concentrations, it is believed that ROS are not harmful to cells and indeed perform useful functions. However, they can be deleterious at high levels, targeting the intracellular substances. The first reactive step is initiated when a molecule of oxygen gains an electron which can come from the electron transport system (Ambrosio et al. 1993, Guidot and et al. 1993) or a phagocytosis (Babior 1978), producing superoxide radical that is not a particularly reactive species but potentially toxic. Subsequently, it can be transformed into hydrogen peroxide, and the hydroxyl radical, when it is not removed by an antioxidant pathway. In the first stage, dismutation, the superoxide radical is converted to hydrogen peroxide. Then, it becomes a hydroxyl radical in the presence of a trace amount of transient metal ions or by interacting with the superoxide radical. Once the reaction starts, hydroxyl radicals initiate the destructive chain reaction, extracting the electron from adjacent substances, which in turn are transformed into new free radicals that damage critical cell components. Their primary target is the double bonds in unsaturated fatty acids in the cell membrane lipids (Pacifici and Davies 1991). This type of reaction will alter the membrane function (Sun 1990) including the loss of fluidity and breakdown of the membrane secretory functions and transmembrane ionic gradient (Barber and Bernheim 1967). The ROS can attack both the sugar and the base in DNA (Kasai et al. 1986), and amino acid
side chains in proteins (Stadtman 1992). In consequence, cell death, mutation, or carcinogenesis can be induced (Cerutti 1985). Furthermore, various chemical residues such as malondialdehyde, the end products of such a lipid peroxidation process, can diffuse away from the site of the chain reaction resulting in cell edema, vascular permeability, inflammation and chemotaxis (Del Maestro 1980).

To protect cells and compounds from this oxidative damage, the body utilizes a defense system in which antioxidants prevent oxidation by converting ROS to less reactive species usually by donating or transferring electrons or hydrogens. The primary defense system is the “sequestration of transient metal”. Some metals in free ion form are believed to react with superoxide radical or hydrogen peroxide, producing the hydroxy radical and hydroperoxy radical which sets up a chain reaction. Therefore, the binding of these metals to transport or storage proteins is important to ensure that these free metals do not react with ROS. Transferrin and ceruloplasmin are good examples of these proteins, which may be important defense systems in the extracellular environment (Machlin et al. 1987). Secondary systems mostly depend on metalloenzymes in which some microelements such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), and selenium (Se) are playing functional roles to limit early ROS formation. Superoxide dismutase in cytosol contains Zn and Cu and in mitochondria contains Mn and converts superoxide to hydrogen peroxide (Fridovich 1983). In a further reaction, hydrogen peroxide is converted to water by two different pathways. GPx, a selenium containing enzyme, converts
hydrogen peroxide to water using GSH as a substrate. Oxidized GSH (GSSG) is then reduced again by glutathione reductase using NADPH as a reducing power. Catalase, an iron containing enzyme found in peroxisomes, catalyzes the decomposition of hydrogen peroxide produced as a result of dismutation of the superoxide radical. These trace elements are not considered antioxidants before they are incorporated into the antioxidant enzymes (Willett 1987). In contrast, in the third system, some essential nutrients directly interfere with the propagation of the reactive chain reaction. Vitamin E, especially alphatocopherol, breaks the chain reaction directly by removing hydroperoxy radical (Tappel 1962). In this reaction, vitamin E donates hydrogen directly to hydroperoxy radical and converts it to hydroxy peroxide, which can be further processed by GPx to become a corresponding alcohol, a less reactive compound. The vitamin E radical then reacts with other radicals forming non-reactive product (NPR) or with dihydroascorbic acid (DHAA) to be converted to vitamin E again. In addition, certain carotenoids such as β-carotene or lycopene exert an antioxidant property by quenching singlet oxygen or other electronically excited molecules. Carotenoids transfer excitation energy from singlet oxygen to their double conjugated bonds returning the oxygen to the ground state. In consequence, the transferred energy in the carotenoid is dissipated through a rotating and vibrating interaction with surrounding solvent. Lycopene, abundant in the tomato, shows potent antioxidant action and is known to prevent LDL
oxidation as well. Vitamin C is an important antioxidant in the aqueous phase and is found in high concentration in certain tissues, especially the eye (Varma et al. 1984).

Selenoproteins

The selenoproteins are proteins whose functions and, therefore, synthesis depend on selenium. Selenocysteine is the 21st amino acid in ribosome-mediated protein synthesis and is the only type of amino acid which is specific for selenium and regulated physiologically (Stadtman 1990).

The mechanism of selenocysteine incorporation into proteins has been established mainly in prokaryotes (Böck et al. 1991). Less is known about the process in eukaryotes, however, many aspects of mechanism have been investigated (Stadtman 1990).

Selenocysteine is synthesized from serine on a unique tRNA, which recognizes the UGA codon in mRNA. The UGA codon, typically known as a stop codon that terminates translation, directs cotranslational incorporation of the selenocysteine residue. Recognition of UGA as coding for selenocysteine appears to be mediated by a secondary structure within the mRNA. In prokaryotes, a conserved stem-loop structure is present immediately downstream of UGA. In addition to the UGA codon, it was found that there were other factors that are required for incorporation of selenocysteine into protein. In E. coli, four gene products were characterized and genes were identified: SelA, SelB, SelC, and SelD (Forchhammer et al. 1989, Forchhammer et al. 1990).
\text{trNA}^{\text{sec}}\text{ is a gene product of SelC and plays a key role in synthesis of selenocysteine. This tRNA is charged with L-serine by seryl-tRNA synthetase to yield seryl- tRNA^{\text{sec}} (Leinfelder et al. 1988). Two gene products of SelA (selenocysteine synthase) and SelD (selenophosphate synthetase) are involved in the next step in which selenium joins the selenocysteine synthesis. Selenophosphate synthetase catalyzes the phosphorylation of selenide (Leinfelder 1990, Glass et al. 1993) producing monoselenophosphate which serves as a donor of selenium (Stadtman 1996). Selenium then replaces the serine residue of aminoacyl-tRNA^{\text{sec}} yielding selenocysteryl-tRNA^{\text{sec}} (Forchhammer and Böck 1991) going through the process in which selenocysteine synthase is involved. In the final stage, selenocysteryl-tRNA^{\text{sec}} is incorporated into a growing peptide chain to produce the selenoprotein in the presence of SELB. This is a gene product of SelB and is a unique elongation factor for selenoprotein synthesis, equivalent to EF-Tu for the other amino acids (Forchhammer et al. 1990).

As noted, much less has been known about selenoprotein synthesis in eukaryotes compared to prokaryotes. However, it appears that there are some similarities in the two systems. It has been reported that tRNA which is aminoacylated with L-serine and has an anticodon complementary to the UGA codon was found in higher organisms (Lee et al. 1989). Although the conserved stem-loop structure following UGA has not been observed in animal systems, a stem-loop structure, the selenocysteine insertion sequence (SECIS) element,
has been described in the 3' untranslated region far downstream on the mRNA (Berry et al. 1991).

Glutathione Peroxidases.

Glutathione peroxidase (GPx) is a selenium dependent enzyme with four distinctive types depending on the compartment: cellular GPx (cGPx), extracellular GPx (eGPx), phospholipid hydroperoxide GPx (phGPx), and gastrointestinal GPx (GPxGI). Each of the four enzymes is different, structurally and functionally. Because the activities of some of them are correlated to selenium in the tissues and are affected by dietary selenium to different degrees, GPx has served as a biochemical index for selenium status and a good parameter for determining the selenium requirement (Sunde, 1994). It is known that GPx activity is subject to numerous factors including dietary selenium intake (Scott and et al. 1977), the type of selenium ingested (Thompson and Robinson 1993), age (Schisler and Singh, 1988), sex (Pinto and Bartley 1969, Igarashi et al, 1983), physiologic status such as pregnancy (Behne and Wolter 1979) and ingestion of certain compounds such as peroxidized lipids (Reddy and Tappel, 1974) or ethanol (Suematsu and et al, 1981).

The best known function of the GPx enzymes in biological system is in their antioxidant property. Together with superoxide dismutase and catalase, GPx is an important antioxidant protecting cells from damage induced by ROS. It catalyzes the reductive destruction of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and a variety of organic hydroperoxides (ROOH) to water or the corresponding hydroxyl
compound (ROH), providing protection against oxidative damage and accumulation of free radical products. It uses glutathione as the substrate and NADPH as the reducing power that converts oxidized glutathione (GSSG) to the reduced form (GSH) with the aid of GSH reductase (Takahashi and et al. 1986). Figure 2.1 illustrates the glutathione peroxidase pathway.

\[
2\text{GSH} + \text{ROOH} / \text{H}_2\text{O}_2 \rightarrow 2\text{GSSG} + \text{ROH} + \text{H}_2\text{O}
\]

Figure 2.1 Glutathione peroxidase pathway

**Cellular GPx (cGPx).** Classical intracellular or cytosolic GPx, cGPx, was the first selenoprotein to be characterized. In 1957 the enzyme was found in erythrocytes where it protected hemoglobin from oxidative damage by \( \text{H}_2\text{O}_2 \) (Mills 1957). However, it was not until 1973 that it was discovered that this enzyme contains selenium as a functional component (Rotruck et al. 1973).

The cGPx unit consists of four identical subunits with a 22 kd (kiodlaton) molecular weight. Each contains one molecule of selenium per subunit with the ionized selenol moiety acting as the redox center in the enzyme (Forstrom et al. 1978). In 1987, Mullenbach et al., who sequenced a GPx cDNA from human kidney, showed that the human gene encodes a 201 amino acid polypeptide with selenocysteine located at the 47th residue (Mullenbach et al. 1987). The cGPx sequence is well conserved in higher animals showing 85% and 90% homologies of amino acid and nucleotide sequence with the mouse. It is
distributed in virtually all cells (Robinson and Thomson 1983) but its specific activity varies greatly depending on species and tissues. In rat tissues, its activity appeared to be high in liver and kidney (Chow and Jeng 1981, Schisler and Singh 1988) with approximately 25% of total cGPx found in the liver (Behne and Wolters 1983). The majority of enzyme activity is present in the cytosol with a small portion in the mitochondrial matrix (Katki and Myers 1980, Aswasthi et al. 1975).

cGPx activity virtually falls to zero in selenium deficiency (Hafeman et al. 1974) and recovers with selenium supplementation (Omaye and Tappel 1974, Chow and Tappel 1974). Generally, the minimal dietary selenium level required for the recovery of the enzyme activity appears to be between 0.02 and 0.1 microgram/gram of diet (Sunde et al. 1989). This dramatic response of cGPx activity to selenium concentrations provides a most useful index for the measurement of selenium status and a parameter for determination of selenium requirements (Sunde 1994). In addition to the decrease in GPx activity, it has been reported that selenium deficiency also leads to a decrease in concentration of cGPx protein to 20% or less (Knight and Sunde 1987, Speier et al. 1985, Takahashi et al. 1986) and that selenium status has an effect on cGPx mRNA levels as well. In rats fed a selenium deficient diet, mRNA levels for cGPx decreased far more than other selenoproteins and was 6-15% of selenium adequate levels (Saedi et al. 1988, Hill et al. 1992), indicating synthesis of the cGPx protein cannot proceed in the absence of selenium. In addition, subsequent studies showed that liver cGPx mRNA (Weiss et al. 1997) and cGPx
protein level (Knight et al. 1988) elevated with an increase in dietary selenium, which preceded an increase in GPx activity. These findings indicate that selenium may regulate the synthesis of this specific enzyme in a unique way.

Although it has been believed that cGPX is involved in regulation of intracellular hydroperoxide concentration, it may occur only when a relatively large amount of H$_2$O$_2$ or lipid hydroperoxide are generated in the cell cytosol. The major role of cGPx as an antioxidant has been challenged by the observation that the removal of H$_2$O$_2$ decreased only 1% in selenium deficiency, which suggests that the primary role of this enzyme may be as storage for selenium (Burk and Hill 1993). Consistent with this idea, several authors have proposed that the cGPx may serve as a selenium reserve which releases selenium to be used for synthesis of other selenoproteins when the selenium supply is limited (Burk and Gregory 1982, Yang et al. 1989).

**Extracellular GPx (eGPx).** Extracellular GPx (eGPx), like cGPx, is a tetrameric enzyme made up of four identical 24 kd subunits with one molecule of selenium per subunit. The enzyme was identified in 1986 (Takahashi and Cohen 1986) and found in breast milk and plasma (Bhattacharya et al. 1988, Avissar et al. 1991). It is responsible for less than 5% of total GPx activity in whole blood (Paglia and Valentine 1967). Although it shares many features with cGPx, including some sequence identity, it appeared to be distinct from cGPx immunologically (Takahashi et al. 1987). Avissar et al. (1989) confirmed this, showing that the antibodies produced from this protein were entirely noncross-
reactive with cGPx. The eGPx is found to be a glycoprotein (Takahashi et al. 1987) as are several other proteins in plasma. It is synthesized in the kidney and the lung but not in the liver (Chu et al. 1992).

The activity of this enzyme decreases in selenium deficiency and increases with enhanced selenium intake, thus serving as a convenient indicator for rapid changes in selenium status (Rotruck et al. 1973). Meanwhile, its activity stays at a maximum with selenium intakes more than 1.0 microgram/gram of diet. This suggests that eGPx might not be a sensitive marker to reflect nutritional status of selenium when dietary selenium consumption is very adequate.

Although eGPx has been considered to have an antioxidant function using glutathione as the substrate, recently, it has been proposed that it may have a different function (Burk and Hill, 1993). This idea is supported by the fact that the extracellular concentration of glutathione, the substrate for the reaction, is only 0.02mM (Anderson and Meister 1980) while the Km for glutathione for this enzyme is in the milimolar range (Avissar et al. 1989, Broderick et al. 1987). These results suggest that eGPx may have broader specificity for substrate or play a different role from cGPx.

**Phospholipid hydroperoxide glutathione peroxidase (phGPx).** Phospholipid hydroperoxide glutathione peroxidase (phGPx) was the third GPx to be identified (Ursini 1985). Although it was identified as a selenoprotein in 1982, it was almost a decade later when it was characterized by Schuckelt et al.
It is a monomer of 20 kd with one selenocysteine residue and is similar to a single subunit of the other two forms of GPx. PhGPx and cGPx are encoded by separate genes with only about 40% amino acid and nucleic acid sequence identity.

PhGPx appears to be more resistant to selenium deficiency, showing a slower decrease in its activity and protein level in comparison with other forms of GPx (Arthur 1992, Guan et al. 1995). In an effort to find out the different regulation for different forms of GPx by dietary selenium, Lei et al. (1985) found that the dietary selenium requirement to reach maximal liver phGPX activity was half that required for maximal cGPX activity. These results suggest that it has a crucial role as an antioxidant and it may not be a good index for selenium status in the body.

Tissue distribution of phGPx is similar to that of cGPx, although the relative amount of phGPx differs from that of cGPx in these tissues. In the rat, it is found in relatively small amounts in the liver whereas it is quite abundant in the testis where it may be under the control of gonadotropins (Roveri et al. 1992). Significant amounts of its activity have also been shown to be associated with the subcellular membrane (Ursini and Bindoli 1987).

It has been well known that cGPx cannot reduce the phospholipid hydroperoxides directly and it requires liberation of oxidized sn-2 fatty acyl groups by phospholipase A2 (PLA2) for its action (Grossman and Wendel 1983). Moreover, cholesterol hydroperoxides are resistant to cGPx even after being extracted from the membrane (Little 1972). In contrast, phGPx, is capable of
catalyzing fatty acid hydroperoxides esterified to phospholipids utilizing thiols such as glutathione to specifically scavenge phospholipid hydroperoxides (Ursini et al. 1982). In addition, several recent works have indicated that phGPx can readily reduce hydroperoxides of cholesterol, cholesterol esters, and phospholipids in low density lipoprotein, thus displaying its broader specificity for hydroperoxides compared to cGPx (Thomas et al. 1990, Sattler et al. 1994).

**Gastrointestinal GPx (GPx-GI).** Gastrointestinal GPx (GPX-GI), similar in properties to cGPX, is a cytosolic tetrameric selenium dependent GPx (Chu et al. 1993). This enzyme has been isolated and characterized by expressing a gastrointestinal GPx cDNA isolated from human hepatoma HepG2 cells in human mammary carcinoma MCF-7 cells (Chu et al. 1993). This cytosolic 22.0 kd protein, appeared to reduce hydrogen peroxide as well as fatty acid hydroperoxides, whereas it did not reduce phospholipid hydroperoxide or cholesterol hydroperoxide efficiently (Esworthy et al. 1993). Unlike cellular GPx, which is present in almost every tissue, GPx-GI is expressed in the epithelium of the gastrointestinal tract. Even though GPx-GI mRNA was found throughout the GI tract, the greatest level of expression of mRNA was found in the ileum and cecum (Chu and Esworthy 1995). Cellular GPx mRNA was not detectable in rat small intestine suggesting that GPx-GI is the major GPx in intestinal epithelium. The existence of GPx-GI in intestinal epithelium implies that it plays a role as an antioxidant to protect the GI tract, which is constantly exposed to oxidative stress by dietary lipid and xenobiotics.
**Iodothyronine Deiodinase**

Thyroid hormone is involved in the regulation of various metabolic processes in all vertebrate species. From the thyroid gland, it is released into the circulation in the form of either 3,5,3'-triiodothyronine (T3) or 3,5,3',5'-tetraiodothyronine (T4), responding to thyrotropin stimulating hormone (TSH) stimulation. There is also a minute amount of the biologically inactive form, 3,3',5'-triiodothyronine (rT3), secreted, that is a potent regulator for thyroid hormone metabolism.

In the circulation, T4, thyroxin, is the principal secretory product showing a ratio of 7:3 with the bioactive form, T3. When the iodine supply is sufficient, the thyroid gland contributes only 10-20% of circulating T3 (Abrams and Larsen 1973) and more than 80% is derived from T4 by 5'deiodination in the non-thyroidal tissue such as liver, kidney, and muscle (Gross and Pitt-Rivers 1952, Engler and Burger 1984). This deiodination is a finely regulated process and catalyzed by various deiodinases. There are three different types of deiodinases (Leonard and Visser 1986) defined in terms of its tissue distribution, reaction kinetics, efficiency of substrate utilization and sensitivity to inhibitors and function: type I 5'-iodothyronine deiodinase (ID-I), type II 5'-iodothyronine deiodinase (ID-II), and type III 5'-iodothyronine deiodinase (ID-III).

It has been found that these three types of deiodinases, the important enzymes regulating the formation and degradation of T3, are identified as selenoproteins containing selenocysteine in their active center. These findings
offered a new research area to the field of selenium study, since it was the first
evidence that selenium plays a physiological role other than in GPx.

**Type I deiodinase.** Type I deiodinase, a homodimer with a 27 kd subunit,
is the selenoenzyme responsible for cleaving one iodine from T4 converting it to
T3. It catalyzes both 5'-(outer ring deiodination) and 5-monodeiodination (inner
ring deiodination) (Braverman and Vagenakis 1979, Chopra et al. 1978) with the
former pathway being preferred (Leonard 1986).

The first evidence of the link between selenium and ID-1 was provided
when Beckett and his colleagues (1987) demonstrated that selenium deficiency
reduced hepatic ID-1 activity and decreased T3 and increased T4 levels in rat
plasma. This finding was substantiated by subsequent work in which a
selenoprotein with the same molecular weight and deiodinase activity was
purified, providing the first evidence that type I deiodinase was a selenoprotein
(Artur et al. 1990a). In an expression cloning study, ID-1 from rat liver was
isolated and identified as a selenoprotein, with each subunit containing one
selenocysteine residue which is essential for the catalytic activity of the enzyme
(Berry 1991a). This study also demonstrated that the mRNA contains a single
in-frame UGA codon specifying selenocysteine at the active site in each
substrate-binding subunit (Berry 1991a). The highest level of ID-1 activity has
been found in thyroid, kidney, liver and brain. However, ID-1 activity in thyroid
appears to be species specific, showing relatively low activity in the thyroid gland
of cattle, pigs goat, deer, and rabbits (Arthur et al. 1993).
As noted earlier, it has been well established that selenium deficiency causes a decrease in ID-1 activity leading to decreased peripheral T3 formation (Arthur et al. 1990a, Behne et al. 1990, Arthur et al. 1990b) as well as rT3 (Beckett et al. 1992, Meinhold et al. 1993). This decrease in enzyme activity was restored when selenium was added back to the selenium deficient diet (Beckett et al. 1989, Arthur et al. 1990b). In a selenium repletion study, Behne et al. (1988) illustrated that administered $^{75}$Se was preferentially incorporated into the hepatic and thyroidal ID-1 compared to GPx. These results indicate that ID-1 is supplied with selenium before cellular GPx under selenium deficiency (Groß et al. 1994) and that the selenium requirement for the production of a sufficient amount of ID-1 is lower than for maximum GPx activity, which is 0.1 mg selenium/kilogram diet (Hafeman et al. 1974). These findings suggest that ID-1 may not be a proper index to measure the nutritional status of selenium especially when selenium intake is relatively high.

Although both ID-1 activity and ID-1 mRNA amount decrease in rat liver during selenium deficiency, mRNA concentrations are maintained at control levels while the ID-1 activity decreases in rat thyroid gland during selenium deficiency (Mitchel et al. 1996). Although some investigators have reported that 5-6 weeks of short-term selenium deficiency lead to the marked decrease of T3 and increase of T4 (Beckett et al. 1987, Arthur et al. 1990b), other researchers reported limited changes in circulating T3 and T4 levels in spite of a significant reduction of hepatic ID-1 activity during a two month long-term selenium deficiency (Meinhold et al. 1993). These results may be explained by a
compensatory mechanism to maintain the optimum level of T3 which resists decreased ID-I activity. The suggested mechanisms responsible for these results are the decrease in the metabolic clearance rate of T3, which was evidenced by the study using radiolabeled T3 (Meinhold et al. 1991); prolonged T3 half-life; and increased gut-reabsorption (Chanoine et al. 1992a). In addition, maintenance of T3 could be explained by unchanged or even slightly increased ID-I activity in the thyroid gland (Chanoine et al. 1992a, Meinhold et al. 1992).

**Type II iodothyronine deiodinase (ID-II).** Type II iodothyronine deiodinase catalyzes only 5’ monodeiodination, converting T4 to T3 or rT3 to 3,3’-diiodothyronine (T2). Unlike ID-I, which is expressed in thyroid gland, kidney and liver, ID-II is mainly found in cerebral cortex (Kaplan and Yaskoski 1980), pituitary, placenta (Kaplan and Shaw 1984), and brown adipose tissue (Silva and Larsen 1983), suggesting that it is responsible for the local production of T3 in these tissues. In addition, ID-II activity appears in some fetal and neonatal tissues (McCann et al. 1984), and ensures the supply of a proper amount of T3 for the brain (Calvo et al. 1990), which suggests the important role of this enzyme during the developmental phase.

Among all three subtypes, ID-II was the last enzyme identified to be a selenoprotein (Davey et al. 1995). ID-II shows several properties that are distinguishable from those of ID-I and ID-III, which earlier suggested that ID-II was not a selenoprotein. ID-II is insensitive to the gold inhibitor, the common
inhibitor for the other two subtypes of enzymes (Berry et al. 1991b), and ID-II activity is not decreased when rat astrocytes are cultured in selenium deficient medium (Safran et al. 1991). Even though ID-II activity was moderately decreased in the brain and anterior pituitary gland of selenium deficient rats (Beckett et al. 1989, Chanoine et al. 1992b), this decrease appeared to result from the enhanced posttranslational inactivation of ID-II secondary to elevated T4 levels in circulation and tissues rather than decreased enzyme synthesis directly due to selenium deficiency. Several researchers, however, have identified and characterized rat and human ID-II cDNA both of which code for selenoproteins that contain a selenocysteine that is highly conserved with ID-I and ID-III, confirming that ID-II is a selenoenzyme (Croteau et al. 1996, Valverde-R et al. 1996).

ID-II activity in rat tissue is regulated mainly by thyroid hormone status. Therefore, in hypothyroidism, its activity is markedly increased (Silva and Leonrad 1985), which is associated with a significant increase in ID-II mRNA expression in brown adipose tissue and the pituitary (Croteau et al. 1996).

Type III iodothyronine deiodinase. Type III iodothyronine deiodinase catalyzes the inner-ring deiodination of either T4 or T3 to produce the inactive compounds rT3 or, T2, which results in the inactivation of these hormones since two inner-ring iodine atoms are necessary for activation of the nuclear T3 receptor (Silva and Larsen 1986). Its activity is high in rat brain, skin, and placenta.
Recently, ID-III was identified as a selenium containing enzyme when St Germain et al. (1994) cloned cDNA for *Xenopus laevis* and demonstrated the presence of an in frame TGA codon encoding selenocysteine. Salvatore et al. (1995) provided the precise evidence that this enzyme in human placenta is a selenoprotein by identifying the presence of an in frame UGA codon at position 144 and the selenocysteine insertion sequence element in the 3′ untranslated region of the mRNA which is required for its expression. This selenocysteine insertion sequence element appeared to be more potent than that in the ID-I or GPx gene, suggesting a high priority for selenocysteine incorporation into this enzyme. ID-III activity in the cerebral cortex decreased in the selenium deficient rat (Leonard et al. 1981) but the effect was limited when compared to the response of ID-I activity to selenium deficiency.

**Selenoprotein P**

Selenoprotein P is a rat plasma protein identified as early as 1977 to be a selenium-containing protein distinct from glutathione peroxidase (Herman 1977). Since then, the selenium in this protein has been found to be a selenocysteine (Motsenbocker and Tappel 1982) and selenoprotein P is the only selenoprotein identified so far to have multiple selenocysteine residues (7.5±1.0) per polypeptide chain of 43 kd (Hill et al. 1991, Read et al. 1990). This unusual composition was confirmed by the cloning and sequencing of its cDNA (Hill et al. 1991). This cDNA contains ten, in frame TGA codons in the open reading frame which specifies for incorporation of up to ten selenocysteine residues per
molecule. Selenoprotein P was purified in rat and human plasma in 1987 and 1994, respectively (Yang et al 1987, Åkesson 1994). In the rat, this glycoprotein has twice as much of the selenium as GPx does (Read et al. 1990). It contributes more than 60% of the total selenoprotein in the plasma and therefore, is the major selenoprotein in plasma. In human plasma, its concentration is about a tenth of the concentration of selenoprotein P in rat plasma. Although it appears that virtually all cells are capable of making selenoprotein P (Burk and Hill, 1994), the primary site for selenoprotein P synthesis and secretion is the liver.

Several nutritional studies have shown that reduced selenium intake, both in animals and humans, results in a decrease in selenoprotein P level (Yang et al. 1989, Hill et al. 1996) suggesting that measurement of selenoprotein P can provide a useful index for assessing nutritional status of selenium. This plasma protein is more resistant to a decrease in dietary selenium intake compared to GPx level, with the mRNA for GPx decreasing prior to that of selenoprotein P in the rat liver (Hill et al. 1992). Burk and Gregory reported that, in rat liver, much more $^{75}$Se was incorporated into GPx than into selenoprotein P in selenium adequate animals, while selenoprotein P contained more $^{75}$Se than GPx did in selenium deficient rats (Burk and Gregory 1982). These results confirmed that selenoprotein P concentration is better maintained than GPx level in selenium deficiency and that its level recovers more rapidly than that of GPx when relatively small amounts of selenium are available.
Although the biological function of selenoprotein P has not been clearly established, evidence is accumulating that selenoprotein P plays a role as an antioxidant, acting as a scavenger of free radicals and other oxidants. An early observation by Burk et al. (1980) demonstrated that the selenoprotein P level increased before the GPx level when selenium deficient rats were repleted with a small amount of selenium. This increase in selenoprotein P gave protection against liver injury by diquat treatment (Burk et al. 1980). It has been suggested that selenoprotein P may also serve as a transport protein of selenium from the liver to peripheral tissues (Motsenbocker and Tappel 1982). This hypothesis has been supported by in vitro observations of the receptors for this protein in various tissues (Gomez and Tappel 1989) and that selenoprotein P appeared in the plasma very rapidly after the administration of selenium, preceding its incorporation into tissues (Burk and Gregory 1982, Motsenbocker and Tappel 1982). This concept has been challenged however, by indications that the selenium is covalently bound to the primary structure of selenoprotein P (Read et al. 1990) and that it should be hydrolyzed before it is taken up by the tissues. In addition, although it is expected that selenoprotein P would release selenium readily during selenium deficiency, selenium status had little effect on the disappearance of $^{75}$Se from selenoprotein P (Burk et al. 1991).
Selenoprotein W

It was 1969 when the presence of a low molecular weight selenium containing protein in ovine muscle and heart was proposed by Pedersen et al. (1969). Since then, further investigations on lambs with white muscle disease, a selenium deficiency myopathy, have provided evidence for this protein (Muth, OH et al. 1958, Pedersen et al. 1972, Black et al. 1978). Selenoprotein W, of little less than 10 kd and containing one single atom of selenium in each molecule as a selenocysteine, has been purified from rat muscle (Vendeland 1993). The major amino acids in this protein were glutamate, glycine, lysine, and valine. Previously, it was called G protein, named after ghost, because of its characteristic to disappear during extraction (Whanger 1987). However, to avoid confusion with the G-protein family acting in signal transduction, this selenoprotein was designated to be selenoprotein W from its association with white muscle disease (Whanger et al. 1993a).

The cDNA for this protein has been sequenced and it has been shown that there is a UGA in the open reading frame corresponding to the insertion of selenocysteine (Vendeland et al. 1995). In a study of 5 different species it was revealed that a TGA encoding selenocysteine is the 13th codon of all the cDNAs and that the encoded amino acid sequences are identical for the rat and mouse proteins, and for the human and monkey proteins (Whanger et al. 1997).

The tissue distribution of this protein appears to be species specific. In rats, it is found in muscle, spleen, testis, and brain, is present mainly in cytosol, and is found in very small amounts in the membrane (Yeh et al. 1995). In
sheep, selenoprotein W level was greatest in skeletal muscles and heart and the lowest in the kidney (Yeh et al. 1997a). In the rat the selenoprotein W level in the heart has been found to be very low even after selenium supplementation (Yeh et al. 1997b). Even though the concentration of selenoprotein W in muscle appears to be low in some species, the total content in the whole body would be significant because muscle contributes about 60% to the body weight. This wide tissue distribution may imply an important metabolic function of the tissue distribution and the level of selenoprotein W is known to be affected by dietary selenium. When rats were fed graded levels of selenium (Yeh et al. 1995), selenoprotein W was undetectable in skeletal muscle of rats fed the basal diet, detectable in those fed 0.1 ppm selenium in the diet, and much higher in muscle from rats fed 4 ppm selenium diet. In subsequent work, when selenium was included in the diet, selenoprotein W was detected in rat tissues such as heart, lung, and kidney, but was not detected when selenium was not added in the diet (Sun 1998). A similar effect was observed in sheep, and demonstrated that selenoprotein W concentrations in different tissues varied with different levels of dietary selenium. These findings suggest that the regulation of selenoprotein W by selenium is different for various tissues (Yeh et al. 1997a). In addition, the dietary regulation of selenoprotein W mRNA has been suggested by showing that selenium supplementation increases muscle mRNA in rat and sheep (Vendeland et al. 1995, Yeh et al. 1997a). Because of this rapid
response to dietary selenium intake, the measurement of selenoprotein W has been suggested to be a useful method of determining selenium status (Whanger et al. 1993a).

Like selenoprotein P, the biological function of selenoprotein W has not been elucidated clearly. It is speculated that it serves as an antioxidant, as do other selenoproteins, since selenium is present as selenocysteine. Moreover, this idea is strengthened by the finding that glutathione binds to selenoprotein W (Beilstein et al. 1996), which may suggest the possible involvement of selenoprotein W in the metabolism of glutathione.

**Sperm mitochondrial capsule selenoprotein**

The mitochondrial capsule selenoprotein has been thought to be a major structural protein of the keratinous mitochondrial capsule, a structure that surrounds the mitochondria in mammalian sperm (Calvin et al. 1981a). Since Rosenfield and Beath (1964) proposed the possible role of selenium in male reproduction the relationship between selenium and spermatogenesis has been further investigated.

In 1978, selenium was identified and localized in a 17 kd cysteine-rich polypeptide in the rat spermatozoon, which suggested a critical role of selenium for normal assembly of the sperm tail (Calvin 1978). In a $^{75}$Se administration study, Brown and Burk (1973) showed that selenium is incorporated into rat spermatozoa and concentrated in the midpiece. The selenium level of the spermatozoa in the rat is about 21 milligram /kilogram dry mass, which, by far,
exceeded the level of this element in other compartments of the rat (Behne et al. 1986). The maximum incorporation of selenium occurs at step 7 and 12 of spermatogenesis and decreases by step 15 (Calvin 1978, Calvin et al. 1987). The early increase in testis selenium during spermatogenesis coincides with the initiation of spermatogenesis, and implies that this increase ensures the adequate supply of selenium for the spermatozoa. Due to its location and proposed function, this protein has been designated as the mitochondrial capsule protein (MCP).

In 1990, Kleene et al. (1990) reported that the major reading frame of cDNA from a mouse testis encoded a 143 amino acid protein containing > 20% cysteine and proline. In a subsequent study, however, it was found that the mouse MCP reading frame begins 54 codons further upstream than previously reported and that these additional codons contain three in-phase UGA codons, which normally signify stop but encode selenocysteine in bacterial and mammalian selenoproteins (Karinpour et al. 1992). This appeared to be the first selenoprotein, besides selenoprotein P, that contains more than one selenocysteine residue for a subunit.

The effect of selenium deficiency on sperm development has been well characterized in animals by a series of studies suggesting that selenium is required for the development of the sperm mitochondrial capsule (Wu et al. 1973, Wu et al. 1979, Wallace et al. 1983). In selenium deficiency, the initial change observed is the abnormal development of the sperm midpiece (Calvin et al. 1981b). When animals are maintained on a selenium deficient diet for
prolonged periods, morphological and functional changes occur. Several authors have reported that the mitochondrial sheath was disorganized in long term selenium deficiency resulting in reduced sperm motility in the mouse (Wallace et al. 1983) and rat (Wu et al. 1973). In addition, a study utilizing light microscopy displayed abnormal breaks and sharp bends in the midpiece of sperm (Wu et al. 1979). It has also been shown that sperm production decreases and testicular size is reduced as a result of selenium deficiency (Wallace et al. 1983).

Although the precise function of selenium in spermatogenesis is still obscure, these accumulating results suggest selenium deficiency may cause infertility at least in some species due to the structural and functional defects in selenium deficiency.

With accumulating evidence of an association of selenium with reproduction in humans and animals, there has been controversy over the identity of MCP with phGPx. A possible role of phGPx, the major selenoprotein in rat testis, in spermatogenesis has been suggested due to the observation that it appears to be gonadotropin-dependent and that it appears after puberty. It has been postulated that MCP and phGPx are the same selenoprotein because phGPx expression is consistent with MCP. However, this possibility is still controversial and there has been a report that the deduced amino acid sequence of phGPx is different from that of MCP (Kleen et al. 1990).
**Selenophosphate synthetase**

During the synthesis of selenoprotein, selenocysteine is co-translationally incorporated into prokaryotic and eukaryotic selenoproteins at in-frame UGA codons. Monoselenophosphate serves as a selenium donor required for production of selenocysteine and seleno-tRNAs and is formed from ATP and selenide (Stadtman 1996). Selenophosphate synthetase, the SelD gene product, is the key enzyme for the synthesis of this selenium donor compound catalyzing phosphorylation of selenol (Leinfelder 1990, Glass et al. 1993).

Recently, unlike *E. coli* selenophosphate synthetase (SPS, the SelD gene product) which is not a selenoprotein, it has been reported that selenophosphate synthetase 2 (SPS2), the molecular cloning of human and mouse homologous of the SelD gene, is a selenoprotein. It appeared to possess an in-frame TGA codon at a site corresponding to the enzyme's putative active site which is *E. coli* cysteine 17 (Guimaraes et al. 1996, Wilting et al. 1997). In addition, Kim et al. (1997) showed the presence of selenocysteine in SPS2, which is indicated by a TGA codon in the open reading frame in a position corresponding to the essential cysteine of the *E. coli* enzyme (Kim et al. 1997). In addition, the replacement of a selenoprotein residue with cysteine drastically reduced enzyme activity, indicating that the selenocysteine residue is essential for eukaryotic selenophosphate synthetase activity.

**Thioredoxin Reductase**

Thioredoxin reductase (TR), an NADPH-dependent flavoenzyme, has been purified and characterized from various tissues and species including, yeast
(Speranza et al. 1973), rat liver (Chen et al. 1978), bovine liver (Holmgren 1977), and human placenta (Oblong et al. 1993). *E. coli* TR is a 68 kd flavoprotein with two 34 kd subunits, whereas mammalian TR is a larger dimeric protein of 58 kd. Recently, human TR, isolated and purified from T-cell and lung adenocarcinoma cell line, has been found to be a selenoprotein (Gladyshev et al. 1996, Tamura and Stadtman 1996), showing the similarity to other mammalian TRs in subunits and molecular weight. Analysis of pure rat and calf thymus TR demonstrated it contains 0.6 selenium atoms per subunit confirming that it is a selenoprotein.

The main function of TR is to maintain thioredoxin in reduced form, which forms an effective system for reduction of protein disulfides (Gasdaska et al. 1995), including ribonucleotide reductase required for DNA synthesis. It appears to have more variety of substrates compared to bacterial TR, which is highly specific for thioredoxin as its substrate (Holmgren et al. 1995). Independent from thioredoxin reduction, it provides reducing power to many other substrates such as selenite (Kumar et al. 1992), selenodiglutathione (Björnstedt et al. 1992, Björnstedt et al. 1995a), selenocysteine (Ren et al. 1993), aloxan (Holmgren and Lyckeberg 1980), vitamin K3, lipoic acid (Luthman and Holmgren 1982) and dehydroascorbate (James et al. 1997). Based on the observation that TR reduces selenite and selenodiglutathione (Björnstedt et al. 1992, Björnstedt et al. 1995b), it has been postulated that it reacts with the active-site selenocysteine residue of plasma GPx, serving as an electron donor. This idea was confirmed by the report that human TR is an efficient electron donor for GPx.
which converts hydroperoxides to the corresponding alcohol (Björnstedt et al. 1994). This finding demonstrated the importance of the physiological role of human TR in the defense system against oxidation, especially under the condition in which the concentration of GSH, electron donor for GPx, is relatively low, such as in plasma.

In selenium deficiency, rat liver and kidney TR activity decreases 4.5% and 11%, respectively, compared to TR activity in control animals, while it is conserved in the brain implying its critical role in the brain (Hill et al. 1997). The decreasing rate of hepatic TR during selenium deficiency was slower than that of GPx but more sensitive than selenoprotein P. With repletion of selenium by injection, it responds more rapidly than glutathione peroxidase but slower than selenoprotein P. This result indicates that synthesis of TR precedes that of GPx when selenium supply is limited.

James et al. (1997) showed that TR reduces dehydroascorbate (DHA) to ascorbate with and without thioredoxin. In selenium deficiency, tissue ascorbate concentration in rat liver decreased by one third, which accompanied a decrease in activities of GPx and TR, demonstrating a role for TR in ascorbate regeneration from DHA. In addition, there was no change observed in the concentration of GSH which typically is responsible for regeneration of ascorbate, suggesting that the reduction of DHA is due to the decrease in TR dependent DHA reductase activity as a consequence of selenium deficiency. The availability of TR and GPx, the only known enzymes containing selenium
involved in the antioxidant processes, ensures the maintenance of reduced concentrations of GSH and thioredoxin, which are important for optimum function of numerous essential cellular processes.

**Metabolism of Selenium**

Selenium in food is usually present in two organic forms; selenomethionine and its methyl derivatives from plants, and selenocysteine from animal products. Selenite and selenate, inorganic forms of selenium, are used in selenium supplements. All forms of selenium are absorbed by human very efficiently (Pyykkö et al. 1988) and it does not appear that selenium absorption is under homeostatic control (World Health Organization 1987, Beath et al. 1937). According to various metabolic balance studies, the apparent absorption rate of selenium in the human body is between 55 and 70% (Levander 1986). Selenium absorption mainly occurs in the duodenum, caecum and colon, but the major site of absorption for selenite is the ileum. Selenomethionine, the main form of selenium found in food, is absorbed by active transportation, using the same carrier system as for methionine (McConnell and Cho 1965), which can be inhibited by the presence of methionine. Although there is not much known about the mechanism of absorption other than that of selenomethionine, it has been reported that selenocysteine and inorganic selenium absorption is a passive process (Oldfield 1992).
Animal and human studies have established that the bioavailability of selenium depends upon the chemical form, which also influences the distribution of selenium in the body. In general, the organic forms of selenium, such as selenomethionine are more readily absorbed than the inorganic form, such as selenite and selenate, with an uptake of 90% for selenomethionine and about 60% for selenite through the gastrointestinal tract (Stewart et al. 1987). In a study using $^{75}$Se selenite in three women, the absorption rate was 70, 64, and 44% of the oral dose (Tomson and Stewart 1974). Therefore, it was suggested that the utilization of selenium varies depending on the chemical form of selenium ingested more than on the total amount of selenium in the diet. However, there are additional interpretations for those observations besides the type of selenium affecting its availability, including that the availability of selenium can be influenced by other factors, such as the type of food ingested, and the presence of other foods or components in the diet. Young et al. (1982) indicated that selenium from plant sources is more readily available than selenium from animal products. In addition, the absorption rate was different when selenium was given alone and when supplied with the other foods, indicating that the presence of other foods may influence the availability of selenium (Sirichakwal et al. 1985). Other components in the diet such as vitamin A, E, and C (Robinson et al. 1985) appeared to increase the absorption rate of selenium. However, when 1 gram ascorbic acid was given with diet, the selenium absorption reached nearly zero (Robinson et al. 1985). The presence of heavy
metals decreased the selenium absorption because of complex formation with selenium (Burk and Hill 1993, Forbes and Erdman 1983).

Absorbed selenium is transported associated with blood protein mainly in the very low density β-lipoprotein (VLDL) fraction and an unidentified fraction located electrophoretically between α₁ and α₂-globulin fractions (Sandholm 1974), and a small amount is transported with other blood proteins. Especially, selenite has to undergo transformation by the erythrocytes in order to be bound by plasma protein, a process which depends on adequate glutathione levels in the cells. It appears that most of the selenium is initially observed to be transported by albumin, after which it moves to the globular fraction (Sandholm 1975). Recently, the possibility of selenoprotein P as a transporter protein has been discussed since it carries selenium from the liver to the other organs of the body (Motsenbocker and Tappel 1982). Selenoprotein P is the major form of selenium in rat plasma and it contributes 5-10% of total body selenium. Even though there is evidence that it is made in other sites of the body, such as heart and kidney (Hill et al. 1993), it is mainly synthesized in the liver to be excreted into the blood. Gomez and Tappel (1989) reported that several tissues contain selenoprotein P receptors for selenium uptake, supporting the idea that selenoprotein P is a selenium transporter. This hypothesis, however, has been challenged by the findings that it is made in sites other than the liver and that the
selenoprotein contains selenocysteine in the primary structure of the protein. To render selenium, this structure must be destroyed which may not be an efficient way to transport selenium.

Selenium transported in a protein-bound form enters the various organs and tissues to be deposited. The metabolic fate of selenium varies according to the total amount and form ingested, and the overall selenium status of an individual (Committee on animal nutrition subcommittee on selenium 1983). Oster et al. (1988) pointed out that the selenium uptake of the kidney is greater at low and adequate dietary selenium intakes and lower if the dietary selenium supply is high, indicating that the kidney plays a special role in selenium balance. When selenium intake is adequate, the concentration in the liver and the kidney is higher than in the other organs. At lower dietary selenium intake, the concentration of selenium in the liver and muscle decreases while selenium level in the kidney remains high (Oldfield 1992). This suggests that the kidney has a saturation level at the expense of the other organs, especially muscle. Selenomethionine promotes greater selenium retention in the body tissues compared to an equivalent intake of selenocysteine, selenite or selenate (Thomson 1998, Milner 1990). However, it does not necessarily mean the selenium in this form is immediately available for functional selenoproteins. It has been suggested that the difference in retention time for different forms of ingested selenium is due to the nonspecific incorporation of selenomethionine into cellular protein (Sunde 1990).
After absorption, the major metabolic fate of all ingested selenium is incorporation into proteins. In general, absorbed selenium is metabolized to selenite (Olson 1970) or further to selenide which appears to be the most important intermediate in the metabolism of both organic and inorganic selenium. Selenide, then is incorporated into selenoprotein or methylated to be excreted.

Selenium is excreted from the body through one of three routes, in urine via kidney, in feces through gastrointestinal tract, in air via lung. Selenium can also be removed through skin, nails, and breast milk (Oster et al. 1988), but these are insignificant excretory pathways. Tri-methylated selenium, trimethylselenonuim, is the main form of selenium to be removed via the urinary pathway (Palmer et al. 1970), which appears to be the major route of selenium excretion when dietary intake of selenium is adequate (Yang et al. 1989). It has been reported that urinary selenium excretion increased with high selenium intake (Thomson and Robinson 1986) and decreased with low selenium intake (Robinson et al. 1973). In a study with rats, Burk et al. (1972) made a point that rats adjust to dietary selenium changes through alteration of renal excretion of selenium. In their study, urinary selenium was related to dietary selenium intake varying from 6% with a low selenium basal diet up to 67% with a 1 ppm selenium supplemented diet, while fecal excretion was maintained constant at about 10% of the dose. However, it was shown that at very low selenium intake, there is a threshold below which urinary selenium excretion remained constant suggesting that urinary excretion provides the regulatory mechanism in selenium metabolism (Burk et al. 1972). The study done by Robinson et al. (1985) supported those
findings showing that New Zealand women with lower selenium status had lower clearance of plasma selenium and excreted a smaller portion of selenium via urine compared to North American subjects. These studies all together suggest that renal regulation of selenium excretion and the consequent conservation of selenium have important implications especially for the population with low selenium status due to low dietary intake.

Excretion through lung and skin are relatively minor pathways when selenium intake level is sufficient (Oster et al. 1988). However, McConell and Roth (1966) indicated that at an excessive level of selenium in the body, excretion through the lung increases, which is characterized by garlic breath due to the pulmonary excretion of the volatile dimethylated selenium metabolites. Methylation appears to be a detoxification mechanism since methylated selenium is known to be less toxic than selenite and is removed rapidly through excretory routes (Tsay et al. 1970).

Fecal excretion of selenium is mainly from the unabsorbed selenium in the gastrointestinal tract and bile secretions (Levander and Bauman 1966). Oster et al. (1988) observed that the selenium amounts excreted with bile are three times higher than the urinary losses of selenium. Enterohepatic reabsorption of selenium from the bile appears to be a significant mechanism of conserving dietary selenium at comparatively low dietary selenium intakes (Dreosti 1986).

The amount and distribution of selenium excretion by different routes varies depending on the form of selenium ingested. When 200 μg of selenium was supplemented in New Zealand women, the urinary selenium excretion was
57% of the dose in the selenate supplemented group and 27% in the selenomethionine group, respectively (Robinson et al. 1997). Similarly, Yang et al. (1989) reported that the subjects who took a selenite supplement excreted approximately twice as much total selenium both in urine and feces as when they were supplemented with the same equivalent of selenomethionine. Women subjects who took 1 milligram of selenium as selenate excreted 81% of the dose whereas less than a third of this amount was excreted when selenite was given (Trian et al. 1992).

**Requirement of Selenium**

A physiological role of selenium was first reported by Rotruck et al. (1973) who showed that selenium was a functional part of glutathione peroxidase (GPx) in rat erythrocytes. Since then, based on the animal data accumulated, it has been suggested that 0.1 microgram/gram of the diet of selenium intake could satisfy the selenium requirement for most species (Levander 1991). However, it was not until 1980 that the first human dietary standard for selenium was proposed by the US Food and Nutrition Board (FNB) on the basis of the estimation from the animal studies. The Estimated Safe and Adequate Dietary Intake (ESADI), is a category reserved for nutrients which were recognized essential, but for which insufficient quantitative data were available to justify a Recommended Dietary Allowance. The range of the ESADI established for selenium in 1980 was 50-200 microgram/day for adults.
Despite this recommendation, there had been no clear-cut symptoms related to selenium deficiency identified in humans until 1979, when a group of Chinese scientists reported the association between low selenium status and Keshan Disease, an endemic cardiomyopathy (Keshan Disease Research Group 1979a). Selenium supplementation of children in a large-scale intervention study had a favorable effect to prevent the disease, which provided the first evidence for a nutritional requirement of selenium. Another study bringing attention to the essentiality of selenium in humans was the work done by van Rij et al. (1979), who observed skeletal muscle pain and tenderness in a patient receiving total parental nutrition (TPN). The symptoms disappeared after administration of 100 micrograms/day of selenium, as selenomethionine, showing that the symptoms were selenium responsive. Since then, considerable investigations have been done demonstrating the nutritional importance of selenium in human health, and leading to the establishment of a Recommended Dietary Allowance (RDA) for selenium in 1989 (National Research Council 1989).

In the trial to investigate the optimum selenium requirement for humans, Yang et al. (1988) performed dietary surveys in regions that were endemic and non-endemic for Keshan disease. It was found that the disease was not reported in the area where the dietary intake of selenium exceeded at least 19 and 13 micrograms/day for men and women, respectively. Subsequent studies using two experimental approaches to estimate the requirement and to derive RDA for selenium followed. One approach involved balance studies that were
carried out in several countries. In the United States, Levander and Morris (1984) reported that the subjects maintained a positive selenium balance while they were consuming 90 and 74 micrograms/day of selenium for men and women, respectively. It also appeared that a selenium intake of 80 and 57 micrograms/day for men and women, respectively, was enough to have zero balance. In New Zealand (Stewart et al. 1978) and China (Luo et al. 1985), where selenium consumption is lower than in the other parts of the world, the selenium intake required to maintain a balance was 24 and 9 micrograms/day. This result suggested that a person who is habitually exposed to a low level of dietary selenium decreases their excretion of selenium to compensate for low intake (Robinson et al. 1985). Using the selenium balance study as a reliable tool to estimate selenium requirements has been questioned since the suggestion was made that humans have a homeostatic mechanism to maintain selenium balance over a range of dietary intakes (Levander 1987). This was supported by the finding that humans remained in selenium balance in spite of vastly different intakes of dietary selenium (Levander 1988). Mertz (1987) pointed out that the balance study is not appropriate for estimation of trace element requirements by suggesting that balance studies estimate the amount of an element in a specified diet that maintains an existing pool size. Levander (1988) also noted that obligatory losses of trace elements are the function of the pool size and that maintaining a large pool requires a higher intake of selenium than maintaining a small pool.
Another more useful approach than the balance study to examine the selenium requirement for humans was the 'depletion-repletion' study. In this type of study, subjects are given a diet very low in the element of interest for a period of time and then repleted with the diet containing a graded quantity of the element. This technique allows the estimation of how much of the element is required to restore to normal any biological changes which resulted from depletion. Yang et al. (1987) used this approach to study the male residents who lived in the endemic area of Keshan disease since the subjects from that region were naturally depleted with selenium due to dietary intakes of selenium as low as 11 micrograms/day. The subjects were given selenium supplementation as selenomethionine with a range of 0-90 micrograms/day, and plasma GPx activity was monitored for several months. The results showed that the GPx activity from subjects receiving 30 micrograms/day of selenium reached a plateau. Therefore, the investigators concluded that the physiological selenium requirement of these subjects was approximately 40 micrograms/day (10 micrograms/day of diet plus 30 micrograms/day of supplement). The RDA in the United States was established based on this estimated requirement of 40 microgram/day, which was modified appropriately to allow for safety and uncertainty factors for each subgroup of the population. First, the difference in body size was taken into account because the average weight of Chinese subjects was 60 kilogram whereas that of a reference American male and female was 79 and 63 kilogram, respectively. A safety factor of 1.3, was applied to the calculation of RDA based on an arbitrarily assumed coefficient of variation of 15
% to take individual variations in selenium requirements into consideration. The resulting RDAs were 70 and 55 micrograms/day for men and women, respectively. For the other age groups, the RDA was extrapolated from the original data from the Chinese study because there has been relatively little information available. Based on body weight plus a growth factor, 10-15 micrograms/day was estimated for infants and 20 micrograms for older children up to 6 years old. An additional 10 and 20 micrograms/day were incorporated into the women's RDA for pregnancy and lactation, respectively. For the elderly, there was no specific data available and the values for their sex-matched group of adults were retained.

With regard to the RDAs for selenium for adults, the typical western diet can easily provide that amount based on a dietary survey done in the US (Pennington et al. 1989). However, consideration should be given for certain subgroups of the US population who are on special dietary treatments such as children with inborn errors of metabolism that are on protein restricted diets (Lipson et al. 1988) or the patients receiving TPN. There have been lines of evidence suggesting a hormonal effect on selenium status (Behne et al. 1978, Smith et al. 1995, Smith and Picciano 1986), which may have some impact on selenium status measurements when hormonal changes occur in the life cycle including puberty, pregnancy, lactation and menopause. Therefore, this characteristic should be taken into account when estimating the requirement of selenium for those specific groups. In addition, further studies are required to
investigate the optimum level of selenium intake to maximize the possible chemopreventive effect of selenium, (Clark et al. 1996) while at the same time avoiding toxic effects.

Assessment of Selenium

Total selenium in the adult male in the eastern USA has been estimated from an autopsy study to be approximately 15 milligram with the range between 13.0-20.3 milligram (Schoeder et al. 1970). The average content of total body selenium in German adult male is about 6.6 milligram (Oster et al. 1988). This value, which is about half of those reported from the USA and close to estimates in New Zealand (Griffith et al. 1973) where selenium content in soil is very low, may reflect the low intake of selenium in that country. Selenium concentrations in different organs are associated with the total amount and the type of selenium in the diet (Committee on Animal Nutrition Subcommittee on Selenium 1983). Selenium concentrations in the liver and the kidney are higher than in other organs when dietary selenium is adequate. With insufficient dietary selenium, selenium concentration in the liver decreases while that of kidney stays high (Oldfield 1992) indicating kidney may play a special role in selenium balance (Oster et al. 1988). Schoreder et al. (1970) reported that about 6.7 milligram, or 46% of the total body selenium is contained in the muscle tissues in typical North Americans. Although the selenium concentration in the skeletal muscle is lower than in other organs such as liver or kidney, it appears to contain the largest
body pool because muscle tissue is present in the largest amount in the body. Therefore, muscle may serve as a major storage compartment for selenium in the body (Whanger et al. 1993b).

In clinical practice, the common methods used to assess the nutritional status of selenium include the estimation of dietary selenium intake, and biochemical analysis of selenium concentration in several body fractions such as whole blood (Thompson and Robinson 1980), plasma (Van Lente and Daher 1992) or serum (Kuroda et al. 1988), red blood cell (Rea et al. 1979), platelet (Campbell et al. 1989), urine (Robberecht and Deelstra 1984), hair and fingernails (Oeschlager and Menke 1969), and toenails (Morris et al. 1983). The accuracy of dietary estimates of selenium is questionable, because selenium content in food greatly varies due to the marked geographical variation in soil content (Gibson 1990) and there is basic difficulty in quantifying the actual amount of food ingested. In addition, many factors influence the bioavailability of selenium in diet (Levander 1983), making it difficult to estimate the actual intake of selenium. In selenium supplementation studies, it has been shown that organic selenium forms such as selenomethionine are more effective to increase the blood selenium concentration more rapidly than inorganic forms including selenite and selenate (Levander et al. 1983, Alfthan et al. 1991, Thomson et al. 1993), while blood GPx activities were much less sensitive to either forms of selenium supplementation (Xia et al. 1992, Thompson et al. 1993). In contrast, platelet GPx activity responded more sensitively to the inorganic form of
selenium than to organic form of selenium (Xia et al. 1992, Thompson et al. 1993), suggesting the nutritional bioavailability of selenium differs for different parameters.

During analysis of selenium levels in body fractions, concentrations of total selenium or specific selenoproteins can be measured. Selenium concentration in serum and plasma, which contains about 75% of the selenium in whole blood (Lombeck et al. 1977), is known to reflect the current intake of dietary selenium acting as an indicator of short-term selenium status (Néve 1991). The proposed reference range of serum selenium, driven by published data, is 0.046-0.143 micrograms/milliliter (Iyengar and Woittiez 1988). However, it is known that serum or plasma selenium level is affected by several factors besides food intake, including age (Milman et al. 1993, Kostakopoulos et al. 1990, Bortoli et al. 1991), various diseases and health condition (Levander et al. 1987), gender (Burguera et al. 1990), smoking (Grandjean et al. 1992), and environmental exposure (Zachara et al. 1987).

Unlike serum and plasma selenium concentration, it is noted that erythrocyte selenium concentration tends to reflect long-term status of selenium, (Thompson et al. 1993), since red blood cells accumulate the element and presumably indicate selenium intake over their 120 day life span. Selenium concentration of platelets has also been used (Levander et al. 1983, Thompson et al. 1993). Platelet is relatively high in selenium concentration and believed to reflect the recent changes of dietary selenium due to its short life span (Lindberg and Jacobsson 1970).
The urinary pathway is the major excretory route of selenium when dietary intake is adequate (Yang et al. 1989), and urinary selenium concentration is affected by dietary selenium intake (Nahapetian and et al. 1983). Hence, urinary concentration of selenium has been considered to be a reasonable index for selenium status (Robberecht and Deelstra 1984) if the urine sample is collected over 24 hr. Since Oelschlager and Menke (1969) proposed that hair and nail selenium concentrations reflect long-term selenium intake, there has been a body of evidence supporting the positive association between these parameters and selenium supplement use and regional distribution of selenium (Flodin 1990, Alfthan et al. 1992). Analysis of hair and nail concentration of selenium provides a non-invasive and convenient method for assessing selenium status in the body. However, there is awareness that certain anti-dandruff shampoos contain selenium, which may remain in hair and invalidate the measurement (Davies 1982). Toenail selenium, which was higher in individuals from South Dakota, a high selenium area, than in nails from New Zealand, a low selenium area, has been noted as a good indicator for selenium status of long-term intake of selenium (Morris et al. 1983).

Selenoprotein P is the only extracellular selenoprotein in plasma besides extracellular glutathione peroxidase (eGPx). Recently, it has been investigated as a useful parameter to reflect selenium status. In a China study, Hill et al. (1996) reported that selenoprotein P concentration was lower in individuals from Dechang, a selenium deficient area, compared to that in subjects who received a selenium supplement. Selenoprotein P level in those subjects were correlated
with GPx activity, the indicator commonly used for selenium assessment. Moreover, concentration of selenoprotein P of subjects from Dechang increased with selenium supplementation showing that this protein should be useful in assessing selenium status in the human body. This result is supported by a similar report from a selenium repletion study on rat (Yang et al. 1989). However, the analytical method for selenoprotein P concentration in human body has not been available for routine use.

Glutathione peroxidase, a functional form of selenium, has been found to be a reasonable index for selenium status (Hoekstra 1975). Selenium in serum can be present in supposedly inactive forms that cannot be incorporated into the active site of eGPx, or bound to heavy metals such as mercury or cadmium so that they become biologically inactive. Meanwhile, measurement of extracellular glutathione peroxidase (eGPx) has been a useful method for assessing biologically active selenium. Because the enzyme activity of eGPx decreases in selenium deficiency and increases with elevated dietary selenium, it can reflect rapid changes in selenium status in the body. Erythrocyte and platelet GPx activity also have been widely used as markers of selenium status. Platelet GPx activity in particular appears to reflect short-term changes in selenium status. In a Finnish study, it has been shown that the concentration of platelet GPx increased two-fold within 2-4 weeks following supplementation with 200 micograms, whereas plasma GPx activity increased by only 20% (Levander et al. 1983). However, GPx activity reaches a plateau over the nutritional range of selenium intake, suggesting it is not a reliable index to assess selenium status.
once enzyme activity is maximized (World Health Organization 1987). This characteristic enables this enzyme to serve as an indicator of the nutritional requirement of selenium (Rea et al. 1979), however. Other factors such as age, sex, and exposure to prooxidants can also influence the activity GPx, complicating the accurate assessment of selenium status (Ganther et al. 1976).

**Deficiency of Selenium**

Since Schwarz and Foltz (1957) showed that selenium prevented liver necrosis in rats, it has been well documented that selenium is an essential trace element in various animal species (Underwood 1977, Ganther et al. 1977). Animals develop deficiency symptoms that vary with different species when selenium is deficient in the diet. White muscle disease (WMD) is a degenerative selenium deficiency disease of the striated muscles, occurring without neural involvement. It affects mainly lambs and calves but other farm animals including horses, goats, rabbits and poultry are also affected (Schwarz 1944). The disease gets its name from a characteristic lightening of the color of muscle, which sometimes is accompanied by calcium deposits. The disease is characterized by muscle weakness, stiffness and muscle deterioration. The incidence of WMD appeared to be high in areas where the selenium content in soil is low such as New Zealand that had 20-30% of WMD incidence, and most of those deficiency symptoms were prevented by dietary selenium (Hogue 1958, Muth et al.1958). In addition, it has been reported that selenium deficiency
results in exudative diathesis in chicks and pancreatic dystrophy in rats (Patterson et al. 1957, McDowell 1992), and liver necrosis or hepatosis dietetica in pigs (Moir and Masters 1970). Based on these findings, selenium supplementation for farm animals has been practiced in several countries such as New Zealand, Finland and USA using various methods including oral treatment, fertilizing the soil, or parenteral injection. Regardless of the methods, it appeared that selenium supplementation improved lamb reproducibility, reduced ewe mortality and increased growth weight in lambs (Meterell et al. 1985).

In humans, selenium deficiency has been associated with two distinctive disease states. In the late 1970s, studies conducted in China provided firm evidence for the important role of selenium in human health. They reported that selenium deficiency in a discrete region in China was associated with an endemic cardiomyopathy, Keshan Disease (KD). Nonendemic selenium deficiency has been reported in some patients receiving total parental nutrition (TPN) with accompanying cardiomyopathy and muscle pain.

al. described a New Zealand patient who was on selenium free TPN for a month and developed muscular pain and tenderness, which made walking difficult. Biochemical analysis indicated that blood selenium concentration and GPx activity were low compared to the average value. All the conditions were rapidly reversed with addition of 100 microgram/day of selenium as selenomethionine to the infusion fluid (van Rij et al. 1979). This study was the first to demonstrate a selenium deficiency syndrome in humans other than KD, suggesting the importance of selenium supplementation in TPN solutions. There have been other reports of muscle problems and myopathy, related to selenium deficiency in several patients on TPN (Brown et al. 1986, Kelly et al. 1988, Osaki et al. 1998). In a number of cases, severe muscular weakness resulted in difficulties in walking and the symptoms were removed after selenium supplementation. In addition, it has been well recognized that cardiomyopathy associated with selenium deficiency in TPN patients can be a potential problem if selenium status is not maintained. In the USA, several studies showed that dilated cardiomyopathy was associated with selenium deficiency in patients on TPN. In severe cases, the patients did not respond to selenium supplementation and postmortem examination of the hearts revealed myocytolysis and replacement fibrosis similar to that seen in KD victims (Johnson et al. 1981, Fleming et al. 1982). Similar results were described in a case report of a 17-year old girl who had been on TPN for 17 months and died after a cardiac arrest secondary to septic shock (Lockitch et al. 1990). In the autopsy, it was found that her heart was enlarged with replacement fibrosis and showed the features typical of KD.
Other researchers have observed the association between selenium deficiency and erythrocytosis, loss of pigmentation of hair and skin (Vinton et al. 1987), and white fingernail bed (Kien and Ganther 1983) accompanied with muscle weakness in children receiving long-term TPN. In addition Kien and Ganther (1983) also found the elevation of serum activities of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and creatinine kinase, indicating cardiac muscle injury as well as hepatic and skeletal muscle damage.

Despite the accumulating evidence, questions have arisen about the varying effects of selenium deficiency on different individuals on TPN. The time to develop cardiac problems has varied depending on the individuals and ranged from 2 weeks (Volk and Cutliff 1986) to 8 years (Quercia et al. 1984) after TPN was initiated. Also, in some patients, the skeletal muscles were targeted without evidence of cardiomyopathy and conversely most cases of cardiomyopathy did not show skeletal muscle symptoms. Furthermore, low blood selenium without apparent clinical manifestations have been reported in a few other patients on TPN (Lane et al, 1981, Shils et al. 1982, Shenkin et al. 1986, Rannem et al. 1993). These apparent differences in the manifestation of selenium deficiency syndromes in TPN patients indicate the possibility of additional factors involved in their etiology. Sepsis may be one such factor since in some cases, sepsis preceded the cardiac failure (Johnson et al. 1981, Stanley et al. 1982). It has been postulated that the decrease in GPx activity due to selenium deficiency may reduce the ability of the host to deal with the toxic oxygen radicals produced during cellular responses ro such an infection.
Another factor may be a concurrent vitamin E deficiency, which can exacerbate the symptoms of the reduced activity of GPx (Schwartz 1967), and make the myocardium more susceptible to the oxidant injury (Lockitch et al. 1990). Vinton et al.(1987) has suggested that the lack of clinical symptoms, even with low serum selenium, may be due to the redistribution of selenium from serum to other compartments of the body such as cardiac and skeletal muscle where selenium is essential for cellular function.

The reason that selenium concentration decreases in certain TPN patients has remained to be identified even though there have been some suggestions. One of the possible explanation is the decrease in absorption or increase in loss of selenium. Buchman et al. (1994) reported that renal homeostasis of selenium was impaired in TPN patients, supporting the latter possibility. In his study, the patients received 40-60 microgram supplemental selenium and appeared to have low serum selenium levels with elevated urinary loss of selenium, indicating that the renal homeostasis for selenium conservation may be impaired in these patients.

It has been well recognized that unsupplemented TPN solutions contain very little selenium, (1 microgram/2000cm³ of TPN formula) (Zabel et al. 1978). To avoid the clinical syndromes related to selenium deficiency in TPN patients especially cardiomyopathy that may be irreversible once it is fully developed, it is important to provide sufficient amount of selenium. Commonly, sodium selenite, selenomethionine, or selenious acid have been used to restore the blood selenium.
selenium in TPN patients (Néve et al. 1986, Baptista et al. 1984, Lane et al. 1987). However, for the selenium, there is still no clear official recommendation for TPN (Gramm et al. 1995) even though the routine supplementation level may not meet the selenium requirements of TPN patients especially with increased metabolic demands on their anti-oxidative system. Vinton et al (1987) recommended a dose of 2 micrograms/kilogram/day for repletion purpose and thereafter 1 micrograms/kilogram/day for a maintenance dose while 3.0 micrograms/kilogram/day of selenium was recommended for replacement by Kelly et al.(1988). However, King et al. (1981) reported that 400 micrograms/day of selenium supplementation for a week, which was followed by 100 micrograms/day for 5 months was necessary to normalize erythrocyte selenium and GPx activities. This suggests that selenium supplementation for certain cases of long-term TPN patients should be increased.

Keshan disease is an endemic cardiomyopathy in China affecting a narrow zone running from Heilongjiang to Yunnan provinces forming a long belt form northeast to southwest. Its incidence is high in hilly and mountainous areas with heavily eroded soils (Tan 1982) and there is an unaffected spot inside an affected region called “safe island” (Keshan Disease Research Group of the Chinese Academy of Medical Sciences 1979b). Affected areas are low in soil selenium and hence, selenium levels in foodstuffs produced in that area were low. As a consequence, the blood and hair selenium concentrations of the residents in the KD area appeared to be low. Compared to the urban residents in the same area, KD has been more prevalent in peasant families in rural
districts who are strongly dependent on food produced locally (Yang et al. 1980). There had been several cases that families who moved from a non-endemic area to an endemic region developed KD in 3 months, whereas residents from an endemic area who were affected by KD did not show fresh cardiac symptoms after they moved outside of the affected area (Ge et al. 1983). These findings indicate that this disease could be biogeochemical condition caused by multiple factors. The most susceptible population to this disease consists of children under 15 years in the north region, children age 2-10 years in the south region, and women of child bearing age, but to a lesser extent. The fatality rate within five years is more than 50% (Cheng and Qian 1991)

The main symptoms of KD are shortness of breath, cough with hemoptysis, edema, and right quadrant pain. Physical signs include cardiac insufficiency, cardiac enlargement, congestive heart failure, cardiac arrhythmias and ECG changes. Pathologically, KD is characterized by multifocal necrosis and fibrous replacement of the myocardium. There are four subtypes of KD: acute, subacute, chronic, and latent (Yang et al. 1988). Acute type shows sudden onset of cardiac malfunction accompanied by pulmonary edema, arrhythmia and fibrous replacement of myocardium. Subacute type, the most common form in children, was characterized by facial edema, slight cardiac dilation and gallop rhythm. Chronic and latent types, both showed cardiac enlargement. Congestive heart failure develops in the chronic type, but not in the latent type which maintains KD normal cardiac function.
Although KD was first reported to have occurred as early as 1907 (Gu et al. 1993), it was the severe outbreak of the disease in 1935 that initiated the first study regarding its epidemiology, clinical signs, and pathology (Apei 1982). There has been substantial amount of research done to elucidate the etiology of KD. In 1973, there was a report showing that selenium deficiency was widespread in habitants of the KD area (Keshan Disease Research Group of the Chinese Academy of Medical Sciences 1974). Results from analysis of hair and blood selenium showed that selenium concentrations in samples from the endemic area were significantly lower than those from the nonendemic area. GPx activity and urinary selenium excretion also appeared to be lower in residents from the KD area compared to people from the nonendemic area. In 1979, the Chinese findings that selenium supplementation prevented KD were first reported in English and attracted wide international attention due to the striking results (Keshan Disease Research Group of the Chinese Academy of Medical Sciences 1979a). In 1974, the first year of their intervention study, the Chinese scientists included children of susceptible age (1-9 years) and divided them into two groups. One group received a placebo and the other one received sodium selenite once a week in a dosage of 0.5 milligram for 1-5 year old subjects and 1.0 milligram for older children. After 2 years, the placebo group was abolished and added to the treatment group since the results showed such an evident effect of selenium supplementation on the reduction of KD incidence. After one year of selenium supplementation, the incidence of KD in the treated group was 2.2 % while the incidence was 13.5 % in the placebo group. When
they finished the study in 1977, there was no KD reported among those children, providing the evidence of the role of selenium in KD etiology.

Even though there has been mounting evidence showing the relationship between selenium status and KD etiology and the definite preventive effect of selenium on the disease, there still have been certain features of the disease that could not be explained solely by selenium deficiency. First, there have not been KD cases reported in New Zealand and Finland where both soil selenium concentration and selenium status in humans are very low. In China, the prevalence of KD showed seasonal and annual variation and; peaked in winter in the northeast and in summer in southern part (Combs and Combs 1986), suggesting the involvement of an infectious agent (Yang 1985).

One suggestion for the etiology of KD combined with selenium deficiency, was that toxicants such as nitrite (Qing and Fan 1991), barium, or mycotoxins in mouldy food induced KD. Certain nutrient deficiencies such as that of vitamin E (Yang 1983, Yang et al. 1988) sulfur amino acids (Zhou et al. 1983, Luo et al. 1987), molybdenum, magnesium and thiamin has been hypothesized to be an etiology also (Yang et al. 1984). None of these hypotheses, however, can adequately explain the etiology of KD. Another possibility that has been given attention is that KD is induced by a viral infection (He 1979). This hypothesis has been supported by the isolation of strains of enteroviruses from KD patients, including coxsackie virus B4, which induce myocarditis. Furthermore, Bai et al. (1980) reported that Coxsackie virus B4 became more cardiotoxic when it was inoculated into selenium deficient mice than when it was given to selenium
adequate mice. In an effort to find the association of selenium with this virus, a series of experiments were performed by a group of researchers. In the first experiment, they tried to observe the simple effect of Coxsackie virus B4 (CV3/20) on selenium deficient and adequate mice. Myocarditis was induced in both groups of mice but selenium deficient mice demonstrated more cardiac damage, suggesting selenium deficiency potentiates the cardiotoxicity of this virus (Beck et al. 1994a). At the next step, viruses obtained from hearts of either deficient or adequate primary hosts were inoculated into a secondary selenium adequate mice. The result revealed that viruses from selenium adequate caused little or no heart damage in 7 days whereas viruses from selenium deficient primary hosts caused severe myocarditis accompanying $10^3$-fold higher in virus titers. In a following study, the effect of selenium deficiency on host response to an amyocarditic strain, Coxsackie B3/0 was tested (Beck et al. 1994b). As expected, this virus caused no heart damage in selenium adequate mice but moderate heart damage in selenium deficient mice. Again, the virus obtained from selenium deficient mice was inoculated into both selenium adequate and deficient secondary hosts and moderate heart damage was observed in selenium deficient animals whereas the selenium adequate mice were not affected. This showed that selenium deficiency allowed a benign virus to become virulent, apparently as a result of a change in the viral genome. In a subsequent sequencing experiment, they found a six point mutation in the virus recovered from selenium deficient mice resembling the wild-type virulent strain, CVB3/20 (Beck et al. 1995). They also repeated the experiment using a vitamin
E-deficient model finding same mutation (Beck et al. 1996), supporting the idea that vitamin E deficiency exacerbates the cardiac injury by the virus (Beck et al. 1994c) and that antioxidant state may contribute to viral virulence. Based on the finding that the antioxidant system function was impaired in KD (Zhu et al. 1989), it can be postulated that the enhanced oxidative stress due to selenium deficiency gives a favorable condition for rapid replication to virus, which in turn increases the chance for mutation. Another proposed mechanism is that either the host immunity is impaired or that cardiomyocytes become vulnerable to viral attack. Even though it is clear that Keshan disease has a multifactorial etiology with a combination of several factors including selenium deficiency and infectious agents (Ge and Yang 1993), the exact cause of disease remains to be investigated further.

Kaschin-Beck disease (KBD), another endemic disease in China, tends to occur in areas which overlap KD belt (The Research Group on Environment and Endemic Disease, Institute of Geography. 1988). KBD is also thought to be a selenium-related disease. It was first discovered by the Russian physician, Urenski, in 1849 and investigated by Kaschin in 1854. However, the detailed study was done by Beck in the first decade of the 20th century. KBD is an ostroarthropathy characterized by a chronic disabling degeneration and necrosis of the joints. Clinically, weakness is followed by joint stiffness and pain and often the enlargement of joints and deformity of limbs occur in advanced case (Yin 1985). When the disease progresses further, shortening of the fingers and long bones occurs, which may cause growth retardation and stunting (Diplock 1987).
Several hypotheses have been suggested for the cause of KBD including calcium deficiency, sulfur deficiency, strontium excess combined with calcium deficiency, and the presence of toxic compounds in food (Yang 1982). However, the findings that KBD is prevalent in low selenium areas and that it has not been found in selenium-rich areas suggests an association between KBD and inadequate selenium status. Indeed, the analysis done in water, soil, food grains and human hair indicated that the KBD is closely related to low selenium status (Li 1989, Wang et al.1987, Fu et al.1984). Oral selenium supplementation has been studied to test the preventive effect of selenium on KBD in various endemic areas. Results from that trial by He et al. (1988) showed that the incidence of KBD was decreased with 1 milligram/week of selenium supplementation from 42.1% in 1983 to 10.7% in 1986 while the control group did not show any changes in incidence. Again, however, there is much evidence indicating that there are several ecological factors apart from selenium deficiency that may cause KBD. Therefore, interacting factors that initiate the pathology of the disease in the presence of selenium deficiency remain to be identified.

Selenium and Diseases

Cardiovascular Disease

Cardiovascular disease (CVD), the leading cause of death in Western countries (Truswell 1992), is a multi-factorial disease associated with genetic and environmental factors. Of these, smoking, hypercholesterolemia and
hypertension are considered to be the three main causes (Gey 1992) contributing to 50-60% of the cases. In addition, an inadequate intake of certain nutrients, including vitamins and essential trace elements, may contribute to the etiology of CVD. Attention paid to the association between selenium and the pathogenesis of CVD originated from observations of blood pressure changes, cardiomyopathy and sudden cardiac death in animals with dietary selenium deficiency (Burk 1978). In humans, a decrease in blood selenium has been associated with congestive heart failure (Bouil et al. 1992) and congestive cardiomyopathy (Oster et al. 1983). In China, a selenium responsive cardiomyopathy, Keshan disease, was reported among the inhabitants of the Keshan province where soil concentration of selenium is extremely low (Keshan Disease Research Group of the Chinese Academy of Medical Sciences 1974). Ecological studies also suggested a lower CVD mortality in high selenium areas than in low selenium areas (Shamberger 1981). In a large prospective study in Finland, Salonen et al. (1988) reported a reverse relationship between blood pressure and serum selenium levels in suggesting a possible role of selenium in heart disease etiology. Evidence from epidemiological studies has indicated an association between selenium deficiency and heart disease as well. In a prospective study in eastern Finland, Salonen et al. (1982) found that serum selenium concentrations less than or equal to 35 microgram/liter were associated with a 6.9 fold increase in the risk of death from ischemic heart disease compared to subjects with selenium levels in serum of 45 microgram/liter or more.
Selenium deficiency has also been shown to affect the several cellular mechanisms that have been related to the etiology of CVD. It is commonly accepted that a mechanism for development of heart disease is the accumulation of atheromatous plaque on the vessel wall of the coronary arteries. When plaque becomes big enough to block the blood supply, necrosis in the myocardium occurs due to a shortage of oxygen, which eventually leads to the pathogenic condition, myoinfarction. It is believed that damage to the arterial endothelium is a prerequisite for the development of plaque (Dodson and Horton 1987). The initial injury is possibly caused by lipid hydroperoxides. Henning et al. (1987) observed that endothelial cells were irreversibly damaged with the increased production of lipid hydroperoxides derived from free radical-mediated oxidation of polyunsaturated fatty acids. It has been postulated that the concentration of lipid hydroperoxides in blood is elevated by low antioxidant status. Once cell injury is initiated by hydroperoxides, lesion formation and platelet aggregation may then take place, promoting accumulation of plaque on the artery wall (Betteridge 1987). Above a certain level, lipid hydroperoxides also inhibit the synthesis of prostacyclin (Moncada et al. 1976), a vasodilator, and cause an increase in thromboxane production. As a result, the inflammatory reaction increases, promoting the growth of plaque, and the vessel becomes stiff which increases blood pressure, all of which contribute to the development of atherosclerosis. GPx, especially phospholipid hydroperoxide GPx, functions to remove lipid hydroperoxides and therefore affect prostaglandin and leukotriene metabolism in platelets and other tissues. During selenium deficiency, GPx
activity is reduced in the platelet and arterial walls (Combs and Combs 1984), and hydroperoxides accumulate in platelets to inhibit prostacyclin synthesis and aggravate the atherogenic effect of lipid hydroperoxides on the artery wall (Singh et al. 1992). In addition, phospholipid hydroperoxide GPx has a protective effect on the endothelial cells of the artery by increasing the resistance of LDL to oxidation. LDL, which contains a large amount of polyunsaturated fatty acids, is susceptible to oxidation (Heinecke 1987). When LDL is oxidized, it stimulates uncontrolled uptake of LDL by the macrophage via a scavenging receptor. These cholesterol filled macrophages are transformed into foam cells attached on the vessel wall (Parathasarathy et al. 1986). Oxidized LDL contributes to lesion formation by exerting a chemotactic signal for circulating monocytes (Quinn et al. 1987), causing endothelial cells to release chemotactic substances, (Berliner et al. 1990), inducing cytotoxic damage to endothelial cells (Hessler et al. 1979), provoking an immune reaction (Palinski et al. 1990), modulating the regulation of vascular tone by interfering with nitric oxide (NO) (Kugiyama et al. 1990, Napoli et al. 1997), promoting coagulation (Aviram 1989, Fei et al. 1993), and interfering with cell to cell signaling. Recently, there have been indications that oxidized LDL induces gene expression of adhesion molecules and cytokines in arterial wall cells (Berliner et al. 1995, Berliner and Heinecke JW 1996, Clinton and Libby 1992). Thomas et al. (1993) investigated the protective role of GPx against the type of cell damage and observed that lipid hydroperoxide content and cytotoxicity decreased when oxidized LDL was incubated with glutathione and Ebselen (selenoperoxidase mimetic). In addition, cells cultured in a medium
without selenium showed lower GPx and phospholipid hydroperoxide GPx activity, and were more sensitive to oxidative injury induced by oxidized LDL compared to cells treated with selenium. Although the causal relationship between the development of CVD and antioxidant status including selenium, needs to be studied further, existing evidence suggests that a low nutritional antioxidant status predisposes the individual to develop atherosclerosis.

Cancer

Cancer, a multifactorial disease, is second to cardiovascular disease as the leading cause of death in United States causing one in five deaths annually (American Cancer Society 1993). Carcinogenesis is a multistage process in which normal cells undergo initiation, promotion, and progression in a stepwise process to transform into a malignant entity, which is affected by numerous factors. Besides genetic factors, environmental factors such as chemicals, viruses, diet or radiation exposure play an important role in cancer incidence (Ozols 1994). Of these, diet has been implicated as a risk factor for cancer in humans and it has been estimated that up to 36% (10-70%) of all cancer is attributed to diet (Doll and Peto 1981). Some dietary components, such as fat, may exert a causative effect on cancer risk. It is reported that a fat consumption of more than 20 % of total diet kilocalories can increase the risk of breast cancer incidence by enhancing the level of circulating estrogen, which has been associated with mammary tumorigenesis. Conversely, other dietary components such as fiber and antioxidant micronutrients, may exert preventive effects. Of
these, selenium, originally considered as carcinogenic, has been recognized as a possible chemopreventive agent. In 1949 Clay et al. demonstrated that 5 ppm of selenium as sodium selenite had a protective role against hepatoma tumor in rats. In humans, Shamberger and Frost (1969) first reported that there was an inverse relationship between selenium levels and cancer mortality. Since then, numerous investigations including animal, clinical, ecological, and epidemiological trials have been focused on the association between selenium and cancer.

In animal studies, several lines of evidence have shown that selenium substantially reduces the incidence of a wide variety of cancers at doses that are well above (3-15 ppm) the physiological requirement (1 ppm) but that do not affect animal growth and health (Medina 1986, Ip 1986). In many model systems, selenium supplementation has been shown to decrease the incidence of chemically and nonchemically (Greeder and Milner 1980, Schrauzer et al. 1976) induced cancers in the lung (Birt et al. 1982), colon (Birt et al. 1982, Reddy et al. 1988), liver (Daoud and Griffin 1980), and mammary gland (Ip 1985, Thompson et al. 1982). The protective effect of selenium on tumorigenesis appears to be the greatest during the induction or early promotion phases (Ip 1981) and maximal effect has been achieved with continuous supplementation (Ip 1981, Schrauzer et al. 1990).

Ecological studies have shown that the soil content of selenium is inversely related to the incidence of certain cancers. For example Maksimovic
et al. (1998) reported that Zlatior in Serbia had a mortality rate of cancer that was lower than the other part of Serbia that had a lower concentration of selenium in soil and human serum.

There is also accumulating evidence from epidemiological investigations that indicates a chemopreventive effect of selenium against various types of cancer. Many of the results of the epidemiological studies are also supported by prospective cohort studies. The cancers known to be associated with selenium concentration include stomach (van den Brandt et al. 1993a), lung (van den Brandt et al. 1993b), colorectal (Nomura et al. 1987, Schober et al. 1987, Psathakis et al. 1988), and prostate (Yoshizawa et al. 1998). In a 3-year follow up study, Ujiie et al. (1988) reported that subjects who had a lower serum selenium concentration showed a higher incidence of cancer, suggesting that the low selenium level in serum may not be a secondary consequence of tumor growth but that it was present before the tumor. The large intervention study by Clark et al. (1996) brought a clearer view for the anticarcinogenic effect of selenium. The study was originally designed to check the recurrence rate of skin carcinoma in patients receiving 200 microgram per day of sodium selenite. Although there was no significant effect of selenium supplementation observed on the recurrence of skin cancer, it was found that the selenium supplement decreased all types of cancers by 47% and lung cancer by 69%. Selenium supplementation was effective in the reduction of prostate and colorectal cancer as well. Unfortunately, there were not enough female subjects to check the
changes in the breast cancer. This first large intervention study suggests a beneficial effect of selenium as a chemopreventive agent for humans at relatively low levels of supplementation.

In spite of the clear evidence of an antitumorigenic effect of selenium, the exact in vivo mechanism has not been defined. The uncertainty is enhanced by the fact that the effects of selenium on carcinogenesis vary depending on the form, concentration, and duration of selenium administration, and animal species, and the type of cancer. All of these factors appear to change the outcome of selenium supplementation. These issues have led to active research on the mechanism by which selenium acts as an anticarcinogenic compound. Several hypotheses have been proposed to explain the antitumorigenic effect of selenium including antioxidant protection against oxidative damage, changing the metabolism of carcinogens, production of cytotoxic metabolites, and inducing apoptosis of the tumor cell.

Since the best known function of selenium is as an antioxidant as a functional part of GPx, it has been postulated that the anticarcinogenic effect of selenium is due to its antioxidant property. If not removed in a proper way, reactive substances produced from the superoxide radical and lipid hydroperoxides may damage DNA when they attack the nucleus, leading to mutation, which may initiate carcinogenesis. Therefore, selenium plays an important role to prevent the initiation of oxidation by removing reactive oxygen species. However, the chemopreventive role of selenium as an antioxidant is
questionable since GPx activity reaches a plateau at a relatively low dietary selenium level, whereas the chemopreventive action of selenium is effective with higher doses (Bermano et al. 1995).

Another mechanism proposed is that selenium may change the metabolism of certain carcinogens. Some carcinogens initiate tumorigenensis by forming a covalent adduct with macromolecules such as DNA in the cell. A high dietary level of selenium, most commonly as selenite or selenocyanates, has been found to reduce the DNA-adduct formation or DNA damage by carcinogens such as DMBA (Ip et al. 1995, Liu and Milner 1992), 2-acetylaminofluorene (Wortzman et al. 1980) and 2-oxopropyl nitrosamine (Laweson and Birt 1983).

LeBoeuf and Hoekstra (1983) proposed that excess selenium may produce a metabolite that is toxic to tumor cells thus exerting the anticarcinogenic activity. Excess selenite enhances the ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the cell. The toxicity of GSSG may mediate this anticarcinogenic effect.

It has been also suggested that selenium inhibits cell proliferation of tumor cells by inducing apoptosis. This hypothesis has been supported by observations of selenite acting as a strong inhibitor of cell growth in vitro. The precise mechanism of the inhibition is not clearly defined (Medina and Oborn 1981, Medina and Oborn 1983). LeBoeuf et al. (1985) reported that selenium treatment as sodium selenite induced a significant increase in the population doubling time of all stage of the cell cycle except mitosis. Lanfear et al. (1994)
observed that selenium compounds inhibit cell growth in vitro and induced apoptosis, displaying the DNA fragment as a "ladder", a typical characteristic. This finding has been confirmed by several other researchers (Thompson et al. 1994, Ronai et al. 1995).

Reproductive Cycle in Females

Menarche – The First Menstruation

Puberty is the stage of transition between childhood and adulthood and is accompanied by dramatic changes physically, physiologically, and psychologically. Before puberty, body size, body composition, and physiology are basically similar in boys and girls. During puberty, the adolescent growth spurt and sex differentiation and development begin. Maturation in the reproductive system occurs as a result of the spurt of secretory and morphological activities of the gonads. These changes are related to sequential events of reactivation of the hypothalamic-pituitary-ovary (HPO) unit, stimulation of the sex organs, and secretion of sex steroids. In the adult female, cyclicity of menstruation is maintained by the HPO axis. In this system, gonadotropin releasing hormone (GnRH) secreted by the hypothalamus stimulates the pituitary gland to release two gonadotropins, luteinizing hormone (LH) and follicular stimulating hormone (FSH) into the circulation. LH and FSH then act on the
ovary to secrete the major sex hormones, estrogen and progesterone. Estrogen in turn forms both a negative and positive feedback loop to control the amount of GnRH and LH secretion. The GnRH pulse generator in the hypothalamus, of which the mechanism remains unknown, initiates this whole procedure. Several studies showed that the GnRH system differentiates and functions at birth and can sustain a quasi level of gonadotropins to that of the adult (Grumbach 1980, Reiter and Grumbach 1982). In childhood, however, its activity is suppressed because the GnRH amount is kept very low by an unknown mechanism (Grumbach and Grave 1974). At pubertal age, the inhibitory influence is removed to restore its normal pulsatile activity, which in turn initiates the process of puberty. This reinitiation of the pulsatile secretion of gonadotropin results in a nocturnal increase in LH secretion, which is replaced by increased levels around the clock in an episodic manner in the later stage of puberty (Boyar et al. 1972).

In spite of individual variations, the onset of puberty usually occurs about between the age of 10 and 12 in females. At this early pubertal age, the first clinical signs of puberty observed are appearance of pubic hair and breast development. The growth of pubic and axially hair is under the influence of androgens produced by the ovary and the adrenal, whereas the development of the breast is under the influence of estrogen secreted by ovary. It is during this period that the estradiol concentration in circulation begins to rise to the values of adult women in early follicular phase (Jenner et al. 1972, Bidlingmaier et al. 1973, Winter and Faiman 1973) suggesting that the ovary becomes active steroidogenically.
Whereas these characteristics are early events that are not very obvious to monitor as signs of the initiation of puberty, menarche, the first menstruation, is a discrete event. It occurs relatively late in puberty, between the age of 12 and 13 years on the average (Marshall 1978). Menarche is associated with the presence of mature follicles in the ovary, which in turn stimulates the endometrium by secreting estrogen. However, there appears to be much variability between menarche and the onset of the ovulatory cycle. In general, there usually is an anovulatory period for 6 months or more after menarche, which is due to the immaturity of the long positive feedback loop of estrogen. In most cases, regular ovulatory cyclicity is not obtained until several years later (Treloar et al. 1967, Döring 1969, Apter et al. 1978, Metcalf et al. 1983). During this period, most girls attain peak height velocity ages from 10.5 to 13 years. Peak weight velocity and menarche occur approximately 6 months and 1 year, respectively, after the height peak.

It has been shown that several factors affect the onset of the first menstruation, thus advancing or retarding menarche. Moderate obesity (up to 30% above normal weight for age) is related to earlier menarche while severe obesity is known to delay the onset of menstruation. Early menarche is also seen in hypothyroidism, bedridden retarded children, and urban residents. The conditions associated with retarded menarche are poor nutrition (Simondon et al. 1997), excessive muscular development and strenuous exercise (Buvat and Buvat-Herbaut 1991, Lindholm et al. 1994). It is reported that obtaining a proper
fat percentage (Lindholm et al. 1995) as well as a critical body weight is necessary for the onset and maintenance of menstrual cycles (Zurlo de Mirotti et al. 1995).

**The Menstrual Cycle**

In the majority of cycling women, the menstrual cycle lasts between 25 and 30 days during the active reproductive years, and the distribution is skewed with 28-30 day. This intermenstrual interval increases at the two ends of the individual's reproductive years: adolescence and transitional period for menopause (Treloar and al. 1967).

During the normal menstrual cycle, there are cyclic changes of circulating hormones including gonadotropin, and gonadal hormones. Functional and structural changes in reproductive tissues including the uterus, oviducts, endometrium, and vagina also accompany these hormonal changes. This process is maintained by the fine regulation of the hypothalamus-pituitary-ovary (HPO) axis through feedback mechanisms.

In conjunction with the pituitary, the hypothalamus is the important site for hormonal regulation of the female reproductive system. The pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus into the hypophyseal portal system, the vascular connection between hypothalamus and anterior pituitary gland, stimulates gonadotropes in the pituitary to secrete two gonadotropins. Luteinizing hormone (LH) and follicular stimulating hormone (FSH) are secreted in a cyclic fashion. At the same time, the ovary is essential
for normal menstrual cycle by generating peripheral signals which in turn exert a feedback mechanism both in a positive and negative way. Responding to gonadotropin, it secretes sex hormones and other substances such as activin and inhibin which modulate the frequency and amplitude of the gonadotropin pulsatile release by a feedback mechanism. Estrogen, the major gonadal hormone, shows the inhibitory effect both on the hypothalamus and pituitary. Wildt and et al. reported (1981) that progesterone at high concentration inhibits GnRH secretion at the hypothalamus level as well. In addition, it appeared that both inhibin and follastatin, the gonadal protein hormones, inhibit FSH secretion (Ying 1988). Along with the negative feedback, estrogen has a positive effect on gonadotropin secretion. The sharp increase in plasma estrogen level during the midcycle is necessary for the LH surge which triggers ovulation. To accomplish this task, the estrogen level should be greater than 200 pg/ml and this elevated concentration of estrogen should persist for at least 48 to 50 hours (Filicori and et al. 1984). In addition, activin has a positive effect on FSH release (Ying 1988). These sequential events of hormone interaction are responsible for establishing the optimum condition for the maturation of the oocyte and ovulation.

The human reproductive cycle is divided into three phases; a follicular phase which is divided into early and late phase, and is the time for follicular growth; an ovulatory phase which is the time for the mature oocyte to be released into the reproductive duct; and a luteal phase, when the corpus luteum secretes hormones preparing for fertilization. The follicular phase begins one day before the initiation of menstruation and terminates at the onset of the mid
cycle LH surge. With the rapid regression of the corpus luteum at the late luteal phase, the concentrations of estrogen and progesterone in the blood decrease, which withdraws the inhibitory effect of estrogen on the secretion of the gonadotropins (Goodman and Hodgen 1983).

In response to FSH, a cohort of follicles is recruited from a nonproliferating stockpile of follicles during the first 4-5 days of the menstrual cycle (Baird 1987). Once recruited, follicles either grow constantly until they ovulate or undergo atresia (Peters and et al. 1975). The next stage, selection, occurs between days 5-7 of the cycle. During this stage one follicle which is destined to ovulate is chosen. The final stage of the follicular phase is dominance, in which the dominant follicle, the best developed follicle, grows and produces sufficient estrogen to suppress FSH secretion to the level below, which is necessary for the growth of the other follicles (Baird 1975).

The ovulatory phase starts with the midcycle LH surge which is initiated during the brief estradiol peak. In the last two days before the onset of the LH surge, the estradiol concentration increases exponentially reaching its peak 24 hr before the LH surge. The average length of LH surge is 48 hr and ovulation occurs 10-12 hr after peak LH secretion (Pauerstein and et al. 1978). Right before the LH surge, estradiol levels fall precipitously while progesterone concentrations begin to rise.

After ovulation, the luteal phase begins, during which the follicle undergoes structural and functional changes transforming into the corpus luteum, a transient endocrine organ. It secretes principally progesterone and estradiol
(Zander and et al. 1956) which prepare the endometrium for implantation of a fertilized ovum. The function of the corpus luteum is dependent on the pulsatile secretion of gonadotropins (Hutchison and Zeleznik 1984) and its activity is highest on day 7-8 after the LH surge coinciding with the secondary rise of estradiol level. In the absence of pregnancy, the corpus luteum undergoes luteolysis 2-3 days before the new onset of menses.

As the corpus luteum wanes, serum concentrations of estradiol and progesterone fall. This change results in the elevation of FSH and LH levels due to the removal of negative feedback by estradiol and progesterone on the gonadotropin secretion. This increase in the gonadotropins initiates the beginning of the next menstrual cycle.

The Menopause-The Last Menstrual Cycle

The term 'menopause' denotes the loss of the menstruation and is the traditional marker of reproductive senescence in women. Approximately one half of all women reach menopause between age 45 and 50 years, about one-fourth before 45 years, and one fourth after age (Di Zerega 1983). The median age at menopause is 51.4 years in the United States, while in developing countries, the median age at menopause is less than 44 years (Tsafriri 1978). It is known that low socioeconomic status and smoking result in menopause at an earlier age, whereas menopause at a later age may occur in women who had their last pregnancy at an older age (Brambilla and McKinlay 1989). In addition, Nagata et al. (1998) reported that there is an association between diet and age at
menopause showing that women who had a higher calcium intake had a later onset of menopause than those who had a lower calcium intake. There are some debates that the age of the onset of menarche has an influence on the age of menopause. Some studies reported that the late onset of menarche is associated with early menopause (Stanford et al. 1987) while other studies found no relationship between onset of menarche and the age at menopause (Sosa et al. 1994, Whelan et al. 1990).

Even though menopause occurs at a specific time, several physiologic changes precede the actual cessation of the cycle by many years. In spite of the profound differences in individual cycle characteristics, the variance of the menstrual cycles tends to decrease gradually after menarche, which normally lasts through the entire reproductive years. About 5 to 10 years before complete cessation of menstruation occurs, the variance of the menstrual cycle starts again. During this period, in spite of continuous or intermittent ovulation, the fertility decreases progressively showing significant variability of intermenstrual intervals as menopause is approached. Some years before the menopause, responsiveness of the ovary to FSH and LH begins to decrease (Sherman et al. 1976). However, at this perimenopausal stage, women still have an ovulatory cycle. At this earlier stage, the cycle usually shortens because of decrease in follicular phase length, which results from elevated FSH secretion from the pituitary gland (Lenton et al. 1988, Metcalf and Livesey 1985, Reyes et al. 1977, Sherman et al. 1976). Before birth, the stockpile of primordial follicles and oocytes in the ovary reaches a peak, decreasing exponentially afterwards. This
decrease is accelerated in the last decade of reproductive life (Faddy et al. 1992, Richardson et al. 1987). As a consequence, the supply of oocytes is exhausted at the time of menopause leading to the decrease in secretion of estrogen (Sherman et al. 1976) and inhibin by the ovary. In turn, the decreased inhibition on FSH resulting from the decreased levels of those hormones elevates FSH level (Richardson 1987, Sherman et al. 1976), which accelerates recruitment and maturation of follicles (Sherman et al. 1976). However there is no concomitant increase in LH level (Fraser et al. 1973) suggesting inhibin might selectively contribute to FSH elevation. At the later stage before menopause, length of cycle is prolonged possibly due to the failure of ovulation (Metcalf et al. 1981, Sherman and Korenman 1975, Sherman et al. 1976) and the cycle is anovulatory in most cases. During this stage, as the follicular reserve pool shrinks and the number of follicles recruited in each cycle decreases, the time required to reach the threshold for the triggering of the long loop estrogen positive feedback increases, thereby, lengthening the cycle. Both LH and FSH in circulation are increased several fold above the level seen in the early follicular phase in premenopausal women (Chakravarti et al. 1977, Scaglia et al. 1976) while low levels of estrogen and progesterone are maintained (Metcalf et al. 1981, Sherman and Korenman 1975). This may be due to the removal of the negative feedback inhibition on the hypothalamus-pituitary system as a result of decreased secretion of estrogen. This increase in gonadotropin secretion lasts for up to decades.
After menopause, ovarian production of estrogen stops and the plasma level is very low (13 pg/ml) (Goodman and Hodgen 1983, Hillier et al. 1981, Welsh et al. 1983, Channing et al. 1982) which is a similar value with that of castration of females. In menopausal women, ovarian removal did not change the concentration of estrogen significantly indicating that the source of circulating estrogen is not the ovary. According to the concept developed by Süleria et al. (McNatty et al. 1979), the possible source of circulating estrogen in postmenopausal women is androstenedione produced by the adrenal gland. Androstenedione is aromatized to estrogen, mostly the biologically less active estrone, at an extraglandular site such as a fat, liver, and kidney. Age and weight influence this extraglandular conversion of androstenedione to estrogen. Heavy women appear to show a higher conversion rate than slender women (Adashi and Hsueh 1982, Zeleznik 981).

The majority of women at menopausal age suffer from several symptoms related to menopause as a result of the decrease in estrogen levels. The major symptoms are vasomotor instability which is manifested as hot flashes (Kronenberg 1987) and profuse sweating, vaginal dryness and irritation, atrophy of the genitourinary tract (Patek 1972), osteoporosis, and decrease in the size of the breast and uterus (Tatyry et al. 1980, Brincat et al. 1985, Ross et al. 1987). Other symptoms include nervousness, anxiety, depression and insomnia. However, not all women experience these symptoms, which may be the result of sufficient extraglandular conversion of estrogen.
Among those symptoms, osteoporosis is one of the most important changes of interest since it has long range consequences for general health. In the female, peak bone mass is achieved between 30 and 35 years of age, after which time bone replacement does not catch up with bone resorption leading to a decrease in bone mass with age. Bone loss is accelerated during the first 3-4 years after menopause during which the annual rate of bone loss is about 1-3% (Riggs et al. 1987). At the age of 70, about 30-50% of bone mass is lost in most women. In postmenopausal women, trabecular bone is most severely affected showing 50% loss with aging, whereas loss of cortical bone begins later and to a lesser degree. This decrease in bone mass results in mechanical fragility and subsequent fracture, which are major causes of mortality and morbidity in those women (Beals 1972). Estrogen is known to have an effect to inhibit osteoclastic activity to reduce bone resorption rate. Therefore, a decrease in estrogen level enhances the risk of osteoporosis in postmenopausal women. Estrogen replacement therapy results in decreased bone resorption (Horsman et al. 1983), thereby reducing the risk of fractures (Ettinger et al. 1985).

Cardiovascular diseases (CVD) are the major causes of death in postmenopausal women (Henderson et al. 1986). Even though there is no solid evidence for the relationship between estrogen and the pathogenesis of cardiovascular diseases (CVD), it has been suggested that estrogen has a protective role in prevention of CVD including atherosclerosis, myocardial infarction, and stroke (Bush et al. 1983). It is known that there is a possible association between estrogen reduction and incidence of atherosclerosis, which
is initiated by the changes in the profile of blood lipids. In premenopausal women, compared to their age-matched male counterparts, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) levels are lower while high density lipoprotein (HDL) levels are higher. After menopause, however, cholesterol, LDL, and VLDL increase and HDL decreases indicating postmenopausal women are at higher risk of CVD than premenopausal women (Matthews et al. 1989). Several studies have suggested that estrogen replacement therapy reduces the risk of death from CVD (Bush et al. 1987, Stamper et al. 1985).

Selenium and Reproductive Cycle in Female

At several stages of the female life cycle, physiological changes occur dramatically, which are mainly associated with the estrogen production, which fluctuates during the life cycle in a long-term and during the menstrual cycle in a short-term. Typically, puberty is the first event when the physical and physiological changes occur under the influence of sexual hormones. In consequence, pregnancy, and lactation, and menopause will be following, during which the changes in hormonal profile have profound impact on woman’s body. Because of the unique physiology at each stage, special attentions are required for nutritional requirements and status during these periods to insure if they are sufficient to support their physiological need. The Recommended Dietary Allowances (RDAs) for adolescence, however, have been established primarily based on data from studies of adult male subjects. The selenium RDA for
different age and gender groups were published in 1989 (National Research Council 1989). The values for each subgroup were extrapolated from the value for male adults based on body weight and growth factors. This technique may yield inappropriate recommendation for females at various stages in the life cycle since the important factors such as sex, hormonal status, and rapid growth rate were not fully considered.

An association between sex hormones and selenium status has been reported by several investigators. Sex-related differences in selenium status have been observed in rats in which liver GPx activity was higher in female rats than in male rats (Finley and Kincaid 1991, Prohaska and Sunde 1993). The observation that liver GPx activity increased in females after the onset of sexual maturation (Pinto and Bartley 1969) supports the theory that estrogen increases GPx activity. In contrast, after castration, male rats showed an increase in liver GPx activity, which almost approached the level of females (Capel and Smallwood 1983, Igarashi et al, 1984) suggesting that male sex hormones may have a suppressing effect on hepatic GPx activity. In addition RBC GPx activity has been shown to be greater in females than in males (Guemouri et al. 1991), whereas renal GPx activity was shown to be greater in males than in female rats (Finley and Kincaid 1991). These results suggest that sex differences influence the distribution of selenium and GPx activity. The effect of sex hormones on selenium status during early adolescence was suggested by the report, showing that sexual maturation had a different impact on the selenium status of girls and boys during sexual maturation (Marano et al. 1991). Serum selenium
concentration in boys decreased during sexual maturation whereas this change
did not occur in girls, suggesting a sex hormone involvement in selenium
status.

A few studies have suggested a relationship between estrogen and
selenium status. Selenium status has been shown to fluctuate during the
menstrual cycle in female subjects (McAdam et al. 1994), and blood selenium
concentrations have been shown to fluctuate during the rat estrus cycle (Smith et
al. 1995). In a study of three generations of women, Chang et al. (1994)
demonstrated that selenium status was lowest in grandmothers, who had lower
estrogen levels compared to their daughters and granddaughters. Changes in
selenium status during pregnancy also suggest an association between
reproductive hormones and selenium metabolism. During pregnancy, it has
been well established that selenium concentration (Behne et al, 1978, Zachara
et al 1993) and GPx activity (Rudolph and Wong 1978, Gun’ko et al 1990)
decrease. Smith and Picciano (1986) observed a decrease in selenium status
during pregnancy that was independent of dietary selenium. It has also been
demonstrated that blood selenium concentrations dropped in pregnant rats and
were restored to normal levels 2 days after delivery or after hysterectomy (Behne
et al. 1978). There has been little information available on the relationship
between female sex hormones and selenium status in humans. Studies of
estrogen treatment on amenorrheic women (Massafra et al 1997) and of oral
contraceptive use (Capel et al. 1981) have shown an elevation in RBC GPx
activity.
Although it has been reported that selenium status decreases in the elderly, results have been contradictory, showing a decrease (Sampson 1987, McAdam et al. 1984), or no changes (Cals et al. 1997) with advancing age. Blood selenium and GPx activities have also been shown to be lower in postmenopausal women than in younger women (Campbell et al. 1989). The proposed reasons for decreased selenium status in the elderly are low dietary intakes (Simakkala et al. 1984) especially of protein and selenium containing food (Bingham et al. 1981, Bunker et al. 1988), impaired absorption or increased excretion of selenium (Lloyd et al. 1983).

Selenium is an integral part of the antioxidant enzyme, GPx, which protects cell membrane lipid against peroxidation during the aging process (Reddy et al. 1981). GPx is known to decrease malondialdehyde, a byproduct of lipid peroxidation which is known to increase in disease states associated with peroxidative changes (Esterbauer 1982). In addition, various studies provide evidence that low blood selenium concentrations are associated with increased risk of cardiovascular disease (Salonen et al. 1982, Salonen et al. 1984, Willet et al. 1983). This feature of selenium is important for postmenopausal women since a decrease in selenium status in postmenopausal women may accelerate the process of aging and increase their CVD risk in combination with an estrogen deficit. There have been reports that estrogen influences the activity of superoxide dismutase, an antioxidant enzyme, (Whiteside et al. 1983), and glutathione, an antioxidant molecule, (Charlotta et al. 1997). These findings
suggest that estrogen has a certain effect on antioxidant system including GPx activity. Based on the earlier research, selenium status appears to be affected by sexual hormones.
CHAPTER 3

SELENIUM STATUS OF FEMALES DURING EARLY PUBERTY

INTRODUCTION

Adolescence is a period that begins with the onset of puberty and during which children go through dynamic changes physically, emotionally, and intellectually. The most distinct changes during this period are attainment of physical growth, and development of functional reproductive organs with secondary sexual characteristics. These developments require good harmony between adequate nutrition and the successive impact of hormones such as growth hormone, insulin like growth factor, and estrogen (Carr 1992). In most girls, adolescence usually starts between the age of 10-12 years, and about 2 years earlier than in boys. Menarche, the first menstruation, occurs at about 12-13 years of age (Marshall 1978), although there are other factors that affect the time of menarche, such as socioeconomic, nutritional and genetic factors (Styne and Grumbach 1986). The growth spurt is attained in girls before menarche, however, (Marshall and Tanner 1969) with peak height velocity occurring at the
11-13 years, followed by peak weight velocity 6 months later. The concentration of estrogen, which has been considered to play a role in obtaining a growth spurt, starts rising during the puberty and continues to gradually increase throughout the whole period of puberty (Jenner et al. 1972, Bidlingmaier et al. 1973, Winter and Faiman 1973). Klein et al. (1994) reported that the estrogen concentration in prepubertal girls as young as 8 years of age was 8-fold higher than that of serum estrogen in boys at a similar age, and suggested that estrogen drives the earlier growth spurt in girls. During early adolescence when girls start attaining peak height velocity, circulating estrogen concentrations approach (7-37 picogram/milliliter) the estrogen level of cycling women at the early follicular phase (30-100 picogram/milliliter) (Speroff et al. 1989).

Because of the dramatic changes in physical growth during the early adolescent period, nutritional requirements and intakes should be monitored carefully during this period to insure that they are sufficient to support optimum growth. The Recommended Dietary Allowances (RDAs) for adolescence, however, have been established primarily based on data from studies of adult male subjects. The selenium RDA for different age and gender groups were published in 1989 (National Research Council 1989). The values for each subgroup were extrapolated from the value for male adults based on body weight and growth factors. This technique may yield inappropriate requirement for girls at this age since the important factors such as sex, hormonal status, and rapid growth rate, were not fully considered. There have been several lines of evidence suggesting sex-related difference in selenium
status (Prohaska et al. 1993, Finley et al. 1991), and other reports have indicated that estrogen influences changes in selenium concentration and GPx activity, a functional biomarker of selenium. Massafra et al. demonstrated that the use of oral contraceptive (1993) and the treatment of amenorrheic women with estrogen (1997) increased the RBC GPx activity. Smith et al. (1984) also observed a fluctuation of selenium status during the estrous cycle in rats. The effect of sex hormones on selenium status during early adolescence was illustrated by the report, which showed that sexual maturation had a different impact on the selenium status of girls and boys during sexual maturation (Marano et al. 1991). Therefore, based on the results of these earlier studies, it appears important to understand how changes in estrogen concentrations, particularly the increase in estrogen and subsequent growth spurt during early adolescence impact selenium requirements and status of young females. In addition, the level of the adequacy of the typical dietary intake of selenium, the primary determinant for selenium status, should be investigated in this population.

In spite of the importance of adequate nutrition, during growth and development the adolescents have often been considered to have inadequate nutrition. In addition eating habits formed during childhood have a potential lifelong effect on adult coronary heart disease status Nicklas (1995), suggesting that adequate selenium nutrition for this early adolescent girls has an implication. Selenium is an integral component of (GPx), an antioxidant enzyme protecting cells from oxidative damages induced by lipid peroxides (Reddy et al. 1981).
Low selenium status has been associated with several diseases such as cancer (Willet et al. 1983) and cardiovascular disease (Salonen et al. 1982). Therefore, it appears important to maintain an optimal selenium status in earlier life, considering the preventive effect of selenium against diseases of which etiology is related to oxidative damage by lipid peroxides. Although many nutritional deficiencies, including that of selenium are prevented by the homeostatic mechanism that regulates absorption, distribution and excretion (Levander 1987), one of control mechanisms for this age and sex specific group appears to be control of growth velocity (Salmenperä 1997).

The purpose of this study was, therefore, 1) to determine selenium status of the females during early puberty but prior to menarche, and 2) to determine if the dietary selenium in these girls during puberty is sufficient to maintain adequate status of selenium during the growth spurt. Early adolescent girls would maintain suboptimum selenium status, which results from the possible low intake of selenium and low estrogen concentration. The result of this study will provide useful reference values of selenium for this age group of female especially when the age effect on selenium status,
METHODOLOGY

Subjects

The subjects for this study were recruited as part of a larger longitudinal project designed to assess the influence of calcium supplementation on bone mass acquisition during puberty. For the calcium study, four hundred and fifty six healthy Caucasian females aged 9-11 years who were all in pubertal stage 2 were enrolled from Columbus area school districts and were from various socioeconomic backgrounds. None of them had reached menarche at the time of recruitment and all were free of any systemic disease. Among those, forty prepubertal girls were randomly selected for this investigation at the baseline visit of the calcium study.

Procedures for this study were approved by the Institutional Review Board of The Ohio State University, Columbus, Ohio and informed consent was obtained from each subject before enrollment in the study (Appendix A).

Sample Collection

Anthropometric and developmental data including age, weight, height, and pubertal stage was obtained. Weight and height of all subjects were measured by trained personnel and body mass index (BMI, kg/m²) was calculated. Pubertal stage was determined by a self-evaluation using corresponding illustrations of breast and pubic hair development by each subject and recorded.
by the research associate (Appendix B). A menstrual cycle record sheet was completed in subsequent years and age at menarche was identified from that record (Appendix C).

Blood was collected by a phlebotomist by venipuncture into polypropylene syringes fitted with stainless steel needles and dispensed into non-heparinized vacutainers. Blood was allowed to clot and then separated into serum and red blood cells by centrifugation. Samples were stored at -70°C until analyzed for selenium concentrations and GPx activities.

Dietary Information

To provide recent dietary intake information, all subjects were instructed to complete a three-day dietary record by a registered dietitian. The dietary record included all the food and beverages consumed during two weekdays and one weekend day before the blood collection day. The dietary data were analyzed with the nutritional software package, Nutritionist III (Version 8.5 for Macintosh, Hearst Corporation: San Buruno, CA). The Food Processor program (enhanced version 7.11, 1998; ESHA Research, Salem, OR) was also used to determine missing values for selenium. Dietary analysis included an average intake of total energy, protein, carbohydrate, fat, selenium, and vitamin A, C, and E.
**Laboratory Analyses**

**Selenium concentration:** Selenium concentration in serum and red blood cells was measured using electron-capture gas chromatography (Varian Model 3300 with electron capture detector, Sugarland, Texas) with Durabond Megabor® DB 225 column (J & W Scientific, Folsom, California) (McCarthy et al. 1981, Smith et al. 1982).

Operating conditions of instrument were as follows: detector temperature, 300 °C; injector temperature, 220 °C; column oven temperature, 190 °C. Nitrogen was utilized as the carrier gas at a flow-rate of 30 milliliters/minute. All the reagents used were analytical grade reagents. Appendix D summarizes the detail procedure of selenium assay.

**Glutathione peroxidase activity:** Glutathione peroxidase activity of serum and red blood cells was determined using hydrogen peroxide by the method of Paglia and Valentine (1967) (Appendix E). Glutathione peroxidase activity was calculated based on the decrease in absorbance at 340 nanometers that was associated with the disappearance of NADPH per minute and expressed in micromol NADPH/gram of protein for serum samples and in micromole NADPH/gram of hemoglobin for red blood cell samples. Protein concentration in serum was assayed by the Lowry method (Lowry et al. 1951) based on the colorimetric biuret method and measured in a spectrometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) at 500 nanometers. Hemoglobin concentration of red blood cell was determined spectrometrically at 540
nanometers (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) using Drabkin reagent. Detail description for the procedure for these assays are provided in Appendix F.

Data Analysis

Means for each variable including serum selenium, serum GPx activity, RBC selenium, RBC GPx activity and nutrient content of the diet were generated using the Statistical Analysis Software (SAS) program (Release 6.12, 1998. SAS Institute Inc., Cary, NC). Pearson’s Correlation coefficients were determined to identify any relationships between selenium intake and selenium and GPx activity in serum and RBC. The relationship among the various selenium parameters in the blood were determined. All the results are presented as mean ± standard error of mean (SEM) and considered to be significant at p<0.005.
RESULT

The anthropometric measurements and developmental characteristics for the forty early pubertal girls who participated in this study of selenium status are shown in Table 3.1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>10.9±0.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>143.7±1.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>38.6±1.4</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>18.5±0.5</td>
</tr>
<tr>
<td>Menarche (year)</td>
<td>12.9±0.2</td>
</tr>
<tr>
<td>Breast Development</td>
<td>1.98±0.06</td>
</tr>
<tr>
<td>Pubic Hair Development</td>
<td>1.59±0.11</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM.

Table 3.1: Anthropometric and developmental characteristics of early pubertal girls. (n=40)

The mean±SEM age of the girls was 10.9±0.14 years with a range between 9.3 and 12.2 years. The mean age at menarche for these girls was subsequently determined to be 12.9±0.15 years, which was 2.3 years after their participation in this study. The mean ±SEM pubertal stage was estimated to be
1.98±0.06 for the breast development and 1.59±0.11 for the pubic hair development. Calculated combined pubertal stage was 1.8.

The values for serum selenium, serum GPx activity, RBC selenium, and RBC GPx activity are illustrated in Table 3.2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum selenium (ng/mL)</td>
<td>108±4</td>
</tr>
<tr>
<td>Serum GPx (U/g protein)</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>RBC Se (ng/mL)</td>
<td>211±7</td>
</tr>
<tr>
<td>RBC GPx (U/g hemoglobin)</td>
<td>15.9±0.9</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM.

Table 3.2: Serum and red blood cell selenium concentrations and GPx activities in girls early pubertal girls (n=40).

Calculation of Pearson's correlation coefficients revealed that selenium intake was not significantly correlated with any parameter of selenium measured in the blood. A weak but significant correlation between RBC selenium and weight (r=0.3154, p=0.0474) was detected, however a stronger correlation was observed between RBC selenium and BMI (r=0.4024, p=0.0100). There were no significant correlations observed among the selenium parameters measured in blood.
The estimation of the daily intake of total calorie, carbohydrate, protein, fat, vitamin E, and selenium is summarized in Table 3.3. Carbohydrate intake contributed about 52.5% of total energy consumption whereas 33.8% of total calorie was from fat, and 14.9% from protein intake, respectively. The mean daily intake of selenium based on the calculation of 3-day diet record was 100.1±4.3 micrograms/day with a range of 44.3 -172.9 micrograms/day.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie (Kcal)</td>
<td>1938±64</td>
</tr>
<tr>
<td>Carbohydrate(g)</td>
<td>255±9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>72.3±3.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>72.9±3.0</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>9.6±1.0</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>100±4</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM.

Table 3.3 Daily dietary estimates for selected nutrients for early pubertal girls (n=40)
DISCUSSION

Selenium concentrations and GPx activities in the early adolescent girls studied were all in the normal range revealing that the selenium status of healthy girls in this age group is adequate. Plasma selenium concentrations were within the range of values reported for healthy adult subjects (Versieck and Cornelis 1989) (53±21 to 161±19 nanograms/milliliter). In contrast, Rossipal and Trian (1995) reported that serum selenium concentrations decreased between 10 and 15 years of age when compared to other age groups, and was possibly due to a greater weight gain during that period. It has been well documented that geographical differences in circulating selenium concentrations exist. Healthy adults from Western Europe have been shown to have 2-3 times lower blood selenium concentrations than those of adults from the US or Canada (Schwarz 1976). Plasma selenium concentrations of the early adolescent girls in this study were greater than the values from girls at a similar age in Austria (53±22 micrograms/liter) (Tiran et al. 1992), and in France (median=66.1 micrograms/liter) (Malvy et al. 1993). In a study done in 5 regions of Finland, Mussalo-Rauhamaa et al. (1993) reported that means of the selenium concentrations in girls (12 years old) ranged from 72±20 to 81±13 micrograms/liter, which also were lower than those of the subjects in the present study. In a subsequent study, after fertilization with selenium, dietary selenium intake increased from 12-23 in 1980 to 69-82 micrograms/day 1986 and serum selenium concentrations increased up to 106±14 micrograms/milliliter, which
were almost identical to our result. These comparisons emphasize the significant effect of dietary selenium on selenium measurements and suggest that it may be inappropriate to compare selenium status between groups if their selenium intake is substantially different.

RBC selenium concentrations which reflect long-term selenium status due to the long life span (120 days) of the RBC (Nève et al. 1985), were also within the range for healthy adult subjects. This result indicates that the young females prior to menarche had dietary selenium intakes sufficient to maintain an adequate RBC selenium concentration. As with plasma selenium, there have been no reported RBC selenium values for this population in the US. However, when compared to values for healthy women reported by Pleban et al. (1987) (141±14 microgram/milliliter), RBC selenium from these early adolescent girls were greater. The range of RBC selenium concentrations of, 177-247 microgram/liter, reported by Snook et al. (1983) for Ohio residents, however includes the range for the subjects in this study. There have been reported difficulties in comparing the results of GPx activity, because of the lack of uniform units (Diplock 1993), and lack of a standardized assay method, causing wide inter-laboratory variability (Litov and Combs 1991). In spite of these difficulties, the RBC GPx activity for the young females in the present study were within the range reported from other studies of young children and adolescents ;from 8.2±0.6 (Lombeck et al. 1977) to 22.5±7.0 (Van Caillie-Bertrand et al. 1986) Units/gram of hemoglobin. Since plasma GPx activity is a sensitive indicator of selenium deficiency (Thompson et al. 1977), changes in this parameter may be
an important index for subclinical selenium deficiency when selenium intake is marginal. Diagnosing a subclinical selenium deficiency is of interest since it has been suggested that low selenium status, even without detectable deficiency symptoms may induce metabolic or histopathological alterations, which may in turn trigger a disease (Salmenperä 1997). In the present study, there was no linear relationship found between plasma selenium concentrations and GPx activity indicating that the subjects were in adequate selenium status. It has been recognized that at above 79 micrograms/liter, plasma selenium is not correlated with GPx activity in serum or plasma (Diplock 1993) since the ability for the synthesis of GPx is saturated at higher selenium concentration (Whanger et al. 1988).

The selenium measurements observed in this study were a reflection of dietary selenium intakes which were more than double the RDA for selenium for this age group, 45 micrograms/day. This selenium intakes may stem from the fact that protein intakes that also exceeded the RDA of 46 grams/day. None of the 40 subjects had selenium intake lower than the RDA except for one who consumed 44.3 micrograms/day of selenium. It has been reported that the habitual diet of adolescents includes food items from the meat/fish/egg group at least once a day (Samuelson et al. 1996), which is rich in selenium. Maintaining adequate selenium intakes at this age appears important to guard against the maintenance of selenium status at the expense of growth velocity. If selenium intake and status are not maintained at adequate levels in young females at premenarcheal stage, the attainment of full growth may be negatively affected.
Together with liver and kidney, RBC is one of the largest storage of selenium in the body, reflecting the long-term intake of dietary selenium. Although plasma selenium has long been considered as a sensitive index of recent selenium intake, the correlation between two variables has not been consistent in various studies. It might partly result from the difficulties in assessing selenium intake because of high variability in daily selenium intake and in selenium content in diet. In this study, there were positive relationships observed between RBC selenium and BMI and weigh, respectively. This result indicates that, with adequate selenium intake, RBC retains more selenium contributing to these parameters of growth during this rapid anabolic phase.

The young females in the present study were healthy and in optimum status both in physical and sexual maturation. Anthropometric parameters measured including height, weight, and body mass index (BMI) were within the normal range reported by Hamill (1977). Puberty and sexual maturation are typically estimated using Tanner stages (Marshall and Tanner 1969), in which pubertal stages are rated from 1 (preadolescent) to 5 (mature). In the present study, Tanner stage was assessed separately for breast (1.98±0.06) and pubic hair (1.59±0.11) development, and a combined pubertal stage (PS) was calculated, by which adolescents were categorized. Subjects in the present study were at early puberty, as indicate by a PS of 1.8. Considering that 75% of girls attain their maximal growth spurt by PS 3 (Marshall and Tanner 1969) and/or by the median age of 11.4 years (Carr 1992), it was clear that these girls were approaching or at the initiation of growth spurt. By subsequent
determination, it was shown that the subjects started their first menstruation at the mean age of 12.9 years, which is almost identical to the median age of menarche, 12.7 years, in the US (Marshall and Tanner 1969).

Early adolescence prior to menarche is the period during which nutritional needs increase to achieve full growth. It has been reported however that the nutritional intake of young people is inadequate based on the consumption of diets high in fat and sugar and low in carbohydrate-rich fiber (Clavien et al. 1996). These patterns have been blamed on frequent snacking and low consumption of vegetables and fruits (Neumark-Sztainer et al. 1998). The results of the dietary analysis in this study demonstrated that the girls were consuming a relatively balanced diet. Although the percent energy from fat which was slightly over 30% at 33.8, it was however much lower than 40%, reported by Clavien et al. (1996) for age matched subjects in Switzerland. In the Bogalusa heart study, Nicklas (1985) reported that the percent of energy from carbohydrate was 13, from protein was 49, and from fat was 39 in young adolescents, which was similar to our result except the percent from protein which was a little greater in the young female in the present study.

In summary, the results of this study showed that the selenium intake of early adolescent girls are adequate to maintain selenium status measurements within the normal range, and sufficient to support the growth spurt. These results have important implications for this population since during this stage period, these young females are developing muscle mass, the largest selenium pool in the body.
CHAPTER 4

THE EFFECT OF THE MENSTRUAL CYCLE ON SELENIUM STATUS MEASUREMENTS

INTRODUCTION

It is well recognized that oxidative damage is associated with the progression of several diseases, including cardiovascular disease (CVD) (Salonen et al. 1982, Salonen et al. 1984, Willet et al. 1983) and cancer (Yoshizawa et al. 1988, Ujiie et al. 1998). Selenium is an integral component of glutathione peroxidases (GPx), which catalyzes the metabolism of lipid peroxides and protects cell membranes against lipid peroxidation (Reddy et al. 1981). Maintaining an optimum level of selenium and GPx, therefore, is important to protect the host from the development of diseases induced by oxidative damage. It is known that the measurement of selenium status in blood is affected by various factors such as dietary intake of selenium, age, smoking, and health status.
An association between sex hormones and selenium status has been reported by several investigators. Sex-related differences in selenium status have been observed in rats in which liver GPx activity was higher in female rats than in male rats (Finley and Kincaid 1991, Prohaska and Sunde 1993). This difference in enzyme activity may result either from an effect of estrogen increasing GPx activity or an effect of testosterone decreasing it. The observation that liver GPx activity increased in females after the onset of sexual maturation (Pinto and Bartley 1967) supports the theory that estrogen increases GPx activity. In contrast, after castration, male rats showed an increase in liver GPx activity, which almost approached the level of females (Capel and Smallwood 1983, Igarashi et al, 1984) suggesting that male sex hormones may have a suppressing effect on hepatic GPx activity. In addition RBC GPx activity has been shown to be greater in females than in males (Guemouri et al. 1991), but renal GPx activity was shown to be greater in males than in female rats (Finley and Kincaid 1991). These results suggest that sex differences influence the distribution of selenium and GPx activity. Marano et al. (1991) reported that serum selenium concentration in boys decreased during sexual maturation whereas this change did not occur in girls, also suggesting a sex hormone involvement in selenium status.

Changes in selenium status during pregnancy also suggest an association between reproductive hormones and selenium metabolism. During pregnancy, it has been well established that selenium concentration (Behne et al, 1978,
Zachara et al (1993) and GPx activity (Rudolph and Wong 1978, Gun’ko et al 1990) decrease. Smith and Picciano (1986) observed a decrease in selenium status during pregnancy that was independent of dietary selenium. It has also been demonstrated that blood selenium concentrations dropped in pregnant rats and were restored to normal levels 2 days after delivery or after hysterectomy (Behne et al. 1978). There has been little information available on the relationship between female sex hormones and selenium status in humans. Studies of estrogen treatment on amenorrhoic women (Massafra et al 1997) and of oral contraceptive use (Capel et al 1981) have shown an elevation in RBC GPx activity.

The concentration of circulating estrogen, the major female hormone produced in the ovary, fluctuates physiologically during the menstrual cycle. Estrogen, which has a hydroxyl group on the phenol ring, can exert an antioxidant effect by inhibiting the consumption of oxygen and lipid peroxidation and preventing the propagation of oxidative chain reaction (Sugioka et al. 1987). It has been shown that estrogen has an certain effect on antioxidant system. Addition to glutathione peroxidase, Whiteside et al. (1983) reported that superoxide dismutase, which catalyzes the conversion of superoxide radical to hydrogen peroxide, was influenced by estrogen. Charlotte et al. (1997) also reported that antioxidant molecule, glutathione, showed variation in breast tissue during the menstrual cycle, suggesting that the antioxidant role of estrogen can be partly mediated by modulation of these antioxidant systems. The effect of the
dramatic estrogen fluctuations during the menstrual cycle on these antioxidant systems, including selenium and GPx, has not been studied systemically.

The purpose of this study was to determine the timing and magnitude of changes in parameters of selenium status in relation to fluctuation of estrogen concentration during the menstrual cycle. The effect of dietary selenium intake on selenium status was also determined at each phase of the menstrual cycle. The research hypothesis for this study was that parameters of selenium status fluctuate coinciding with the physiological variation of estrogen concentration during the menstrual cycle in premenopausal women. The results of this study will provide information essential for the accurate assessment of selenium status in premenopausal women. If selenium parameters in blood fluctuate during the menstrual cycle, this within individual variability would impact the results and interpretation of studies on the assessment of selenium status of women at reproductive age.
METHODOLOGY

Study Design

This study was designed to assess female selenium status throughout the menstrual cycle. Plasma and red blood cell selenium and GPx activities were determined at four different time points during three phases of the menstrual cycle: once in the early follicular phase (EF: menstruation day 1-3); twice in the periovulatory phase (PO) (E-1; One day before estrogen peak, E; Day of estrogen peak), and once in the mid luteal phase (ML: 7-9 days after ovulation). These time points were chosen because they represent the phases of the cycle in which estrogen levels are the lowest, the highest, and at a mid point, respectively.

Subjects

Subjects were recruited by advertisement (Appendix G) on The Ohio State University campus between August and October, 1997 and included students and employees of The Ohio State Medical Center. The study population consisted of 14 healthy normally menstruating women aged 21-39 years with regular menstrual cycles that were 26-30 days in length.

Inclusion criteria for the study were the following: 1) Maintaining regular menstrual cycles of 26-30 days with the cycle length for the three prior months not changing by more than 2 days; 2) Not receiving any type of prescribed medication or oral contraceptives at least 1 year prior to study; 3) Maintaining a
stable body weight with a desirable body mass index (18 – 25 kg/m²);
4) Abstaining from alcohol or drinking less than 6 drinks/week or less than 2
drinks/day; 5) non smoking or dieting; 6) No past or ongoing chronic illness;
7) Not pregnant or lactating in 1 ear prior to enrollment in the study:  ad 8) Not
exercising for more than 60 min/day or 7 hours/week. Procedures for this study
were proved by the Institutional Review board of The Ohio State University,
Columbus, Ohio and informed consent was obtained from each subject before
enrollment in the study (Appendix H).

Subjects were studied for one menstrual cycle. Preceding the study,
participants were informed about the purpose of the study, prescreened for
menstrual cycle information, existence of ongoing disease and medication
histories by completing the personal information sheet (Appendix I) and medical
history (Appendix J), and were instructed on how to record the 3 –day dietary
intake (Appendix K). Subjects were asked to record all food and beverages
consumed for a three-day period that immediately preceded the blood collection
for each visit. Subjects were instructed to maintain the usual dietary pattern and
activity level. Subjects were asked to bring the completed three-day dietary
records to each visit and any ambiguous dietary information was clarified in
person and/or by the phone. Height and weight were recorded and body mass
The body mass index (BMI, kg/m\(^2\)) was calculated. The total number and the time of the visits varied depending on the duration of the menstruation and the timing of the estrogen peak.

Visits were made and blood samples were collected during the EF, PO (E-1, and E), and ML phases of the menstrual cycle. For the EF phase blood, samples were obtained between 1-3 days after menstruation started. In order to detect the day of the estrogen peak, which is followed by the luteinizing hormone (LH) surge, the subjects were asked to use a commercially available urinary LH kit (Quick & Simple, New York, NY) every morning. The subjects were instructed to use the test stick with the first urine in the morning and not to urinate at least 6 hours before the test. They started using the kit on day 9 after the onset of menstruation and continued to use it until the next day of the LH surge. Blood drawing for the PO phase began on the morning of menstruation day 11 and continued every morning until the day of or a day after the LH surge. Blood from two days before the LH surge was considered to be the sample for E-1, and blood for E was obtained from one day before the LH surge. To confirm the LH surge, the subjects brought the tested strip from the kit to the lab every morning to be examined. The ML phase blood was drawn between 6-8 days after the LH surge. Follow up with each subject confirmed that the next cycle began 4-6 days after the ML phase blood collection.
**Sample Collection**

During each visit, a total of 10 milliliters of 6-hour fasting blood samples was obtained by venipuncture using heparinized vacutainer tubes for trace element analysis (Becton Dickinson, Rutherford, NJ). Blood collections for each subject were performed at approximately the same time of the day throughout the study period to reduce the variability within each individual. Immediately after the collection, plasma and red blood cells were separated with refrigerated centrifugation at 4°C (3000 x g for 20 min) and each fraction of blood was premixed using a vortex before they were divided into several aliquots. Samples were placed into polypropylene tubes equipped with screw-caps containing ethylene propylene O-rings to minimize dehydration during the storage. The tubes and screw caps were prepared by soaking in a 10% acid bath for 24 hours and rinsing four times with double distilled and deionized water. Hemolyzed samples were discarded and samples were stored at -70°C until analysis.

Information from each set of dietary records was analyzed for selenium, total energy consumption, protein and carbohydrate contents using the Food Processor program (enhanced version 7.11, 1998; ESHA Research, Salem, OR). Dietary intake patterns including intake of total energy, protein, carbohydrate and selenium were determined for each of the three phases of the menstrual cycle.
Laboratory Analyses

Selenium levels: Selenium concentration of plasma and red blood cells was measured using a spectrofluorometer by the modified method of Kho and Benson (1982) (Appendix L). The operating conditions for the spectrofluorometer equipped with a UV lamp were as follows: excitation wavelength: 369 nanometers; emission wavelength: 525 nanometers. Selenium content in the samples was manually calculated using values from sodium selenite standards.

Glutathione peroxidase activity: Glutathione peroxidase activity of plasma and red blood cell was determined using hydrogen peroxide by the method of Paglia and Valentine (1967) (Appendix E). Glutathione peroxidase activity was calculated based on the decrease in absorbance at 340 nanometers that was associated with the disappearance of NADPH per minute and expressed in micromole NADPH/gram of protein for plasma samples and micromole of NADPH/hemoglobin for red blood cell samples. Protein concentration in plasma was assayed by the Lowry method (Lowry et al. 1951) based on the colorimetric biuret method and measured in a spectrometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) at 500 nanometers. Hemoglobin concentration of red blood cell was determined spectrometrically at 540 nanometers (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) using Drabkin reagent. Detail description for the procedure for these assays are provided in Appendix F.
Endocrine Test; Estradiol concentration of plasma was analyzed by a radioimmunoassay using a commercially available estradiol kit (Diagnostic Products Corp., LA, CA).

Data Analysis

Analysis of Variance (ANOVA) for repeated measurements was performed to detect the possible differences in outcomes for the three different stages of the menstrual cycle. If a significant difference was noted, a paired t-test was applied to determine the point of difference. The relationships between plasma estradiol and plasma selenium, plasma GPx, RBC selenium and RBC GPx were analyzed using a random coefficient regression model taking into account the different intercept and slope of each individual. Since multiple observations were made on the same subjects, a random coefficient model was an effective analysis of covariance for each individual, avoiding the problem of treating the observations independently, which would more likely result in significance by increasing the sample size. Mean ± standard error of the mean (SEM) was used to express all the data. Results analyzed were considered to be significant at p<0.05.

RESULT

Of the thirty-five women who responded to the study advertisement, fifteen women met the eligibility criteria and entered the study. The respondents who were rejected were either exercising more than 6 hours a week (n=2), taking medications which are known to interact with trace elements including selenium (n=2), had a BMI (Body Mass Index) greater than 27 (n=1), taking oral contraceptives (n=12), or had an irregular menstrual cycle (n=3). Among the fifteen subjects who were enrolled, fourteen women completed the study and one subject who was found to have an anovulatory cycle dropped out. Anthropometric data including age, weight, height, BMI, and the length of the menstrual cycle are shown in Table 4.1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>29.6±1.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157±6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.9±2.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3±0.6</td>
</tr>
<tr>
<td>Cycle length (day)</td>
<td>28.7±0.2</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM.

Table 4.1: Baseline characteristics of premenopausal women at enrollment (n=14).
Figures illustrates the pattern of estradiol (Fig.4.1), plasma selenium (Fig.4.2), plasma GPx (Fig.4.3), RBC selenium (Fig.4.4) and RBC GPx (Fig.4.5) observed over the four time points during a menstrual cycle. The pattern for all the parameters, except RBC selenium, fluctuated over the menstrual cycle and resembled that of estradiol concentration. 17-β estradiol concentration was the lowest during the early follicular phase (EF) and the highest during the periovulatory phase (PO:E). The actual values of estradiol and selenium measurements at four different time points of menstrual cycle are shown in Table 4.2.

<table>
<thead>
<tr>
<th></th>
<th>EF</th>
<th>PO</th>
<th>ML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-1</td>
<td>E</td>
<td>E-1</td>
</tr>
<tr>
<td>17-β estradiol (pg/mL)</td>
<td>45±7^a</td>
<td>189±12^b</td>
<td>247±18^c</td>
</tr>
<tr>
<td>Plasma Se (ng/mL)</td>
<td>112±4^a</td>
<td>124±5^b</td>
<td>128±4^b</td>
</tr>
<tr>
<td>Plasma GPx (U/g protein)</td>
<td>3.8±0.3^a</td>
<td>5.0±1.3^b</td>
<td>5.5±0.3^c</td>
</tr>
<tr>
<td>RBC Se (ng/mL)</td>
<td>221±10</td>
<td>221±11</td>
<td>215±8</td>
</tr>
<tr>
<td>RBC GPx (U/g hemoglobin)</td>
<td>16±1^a</td>
<td>20±1^b</td>
<td>21±1^b</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM. Values in the same row not sharing the same superscript are significantly different at p<0.05

Abbreviations: EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.

Table 4.2 : 17-β estradiol and selenium status measures at four different time points in three phases during the menstrual cycle.
Fig. 4.1. Concentrations of plasma estradiol measured at four time points during three different phases of the menstrual cycle (n=14) (mean±SEM).

* Significant difference between various time points at p<0.0001.
1. EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.
2. SEM=Standard Error of Mean.
1. EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.

2. SEM=Standard Error of Mean.

Fig. 4.2. Concentrations of plasma selenium measured at four time points of three different phases during the menstrual cycle (n=14) (mean±SEM).

* Significantly different from PO and ML at p<0.005. ** Significantly different from EF and ML at p<0.005. *** Significantly different from PO and EF at p<0.005.
Fig. 4.3. Plasma GPx activities measured at four time points of three different phases\(^1\) during the menstrual cycle (n=14) (mean±SEM\(^2\)).

* Significant difference between various time points at p<0.005.

1. EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.
2. SEM=Standard Error of Mean.
Fig. 4.4. Concentrations of RBC selenium measured at four time points of three different phases during the menstrual cycle (n=14) (mean±SEM).
Fig. 4.5. RBC GPx activities measured at four time points of three different phases1 during the menstrual cycle (n=14) (mean±SEM2).

1. EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.
2. SEM=Standard Error of Mean.

* Significantly different from PO and ML at p<0.005. ** Significantly different from EF and ML at p<0.005. *** Significantly different from PO and EF at p<0.005.
Estradiol concentrations at the day of estrogen peak (E), one day before the LH surge, were 5.5-fold higher than those during the EF phase \( (p<0.0001) \) and decreased about 50% during ML phase \( (p<0.0001) \). At the day before estrogen peak (E-1), estradiol levels were 76% of the value at the estrogen peak day demonstrating the sharp increase in estrogen concentrations during periovulatory phase. Likewise, plasma selenium concentrations were significantly greater in the PO \( \text{E-1}: 124\pm5 \text{ ng/mL}, \text{E}: 128\pm4 \text{ ng/mL} \) than in EF \( (112\pm4 \text{ ng/mL}) \) \( (p<0.05) \) and ML \( (115\pm6 \text{ ng/mL}) \) \( (p<0.05) \). In contrast to the changes in plasma selenium levels, mean concentrations of RBC selenium did not vary significantly across the menstrual cycle with any evidence of menstrual cycle phase effect.

Plasma GPx activity was also greater at the day of estrogen peak \( (5.5\pm0.3 \text{ U/g protein}) \) compared to ML \( (4.4\pm0.3 \text{ U/g protein}) \) and EF \( (3.8\pm0.3 \text{ U/g protein}) \), respectively \( (p<0.05) \). Plasma GPx activity in ML and a day before estrogen peak was significantly higher than that in EF \( (p<0.05) \). Cyclic changes in RBC GPx activity followed a similar pattern and magnitude of changes as those observed for plasma GPx activity. During EF, GPx activity in RBC was lower \( (16\pm1 \text{ U/g protein}) \) than that of any other phase and was greatest at the day of the estrogen peak \( (21\pm1 \text{ U/g protein}) \) \( (p<0.04) \).

Analysis with the random coefficient regression model revealed population mean slopes of line fitting estrogen and plasma selenium \( \text{coefficient; 0.0658, p<0.005} \), plasma GPx \( \text{coefficient; 0.0073, p<0.0001} \), and RBC GPx \( \text{coefficient; 0.0241, p<0.0001} \) was positive. There however, was no linear
relationship observed between selenium intake and plasma selenium, plasma GPx, RBC selenium and RBC GPx.

Table 4.3 summarizes dietary intake data computed from the 3-day food records collected during each phase of the menstrual cycle.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>EF</th>
<th>PO</th>
<th>ML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E-1</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Calorie (Kcal)</td>
<td>1670±82</td>
<td>1624±114</td>
<td>1655±107</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>244±15</td>
<td>222±12</td>
<td>228±13</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>60.0±3.8</td>
<td>59.8±4.7</td>
<td>58.6±3.9</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>73.4±4.6</td>
<td>79.4±5.0</td>
<td>84.9±5.1</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM.
Abbreviations: EF; Early Follicular phase, PO; Periovulatory phase (E-1; One day before estrogen peak, E; Day of estrogen peak), ML; Mid Luteal phase.

Table 4.3: Daily dietary estimates for selected nutrients at four different time points in three phases during the menstrual cycle (n=14).

Repeated measures ANOVA did not detect any significant differences in intake of total calorie, protein, carbohydrate, or selenium intake over the menstrual cycle.
DISCUSSION

Although several investigations have suggested that sex hormones are involved in the regulation of GPx activity, the present study is the first in which blood selenium content and GPx activity were measured simultaneously to detect phase-related changes during the menstrual cycle, monitoring the effect of dietary selenium intake on selenium status at the same time. The result of this study demonstrates that all the selenium measurements in blood except RBC selenium show distinct variations during the menstrual cycle in healthy young women. Plasma selenium, plasma GPx activity, and RBC GPx significantly increased from the lowest level during the early follicular phase to a maximum level during the periovulatory phase, which coincided with the peak in estradiol concentration. Thereafter, the selenium parameter values decreased to a middle level during the mid-luteal phase in which estradiol concentration reached the second peak. The time of greatest change for each parameter was between the early follicular phase and periovulatory phase, which was defined as the time of the urinary LH surge. The LH surge, occurs about 14-24 hours after the estrogen peak and 10-12 hours before ovulation, attaining the peak level about 18 hours before ovum release (Guyton 1986), and provides an accurate physiological marker of the time of estrogen peak and ovulation (Ross et al. 1970). The overall pattern and mean values of plasma estrogen concentrations
observed in the study were also in agreement with results previously reported (Muneyyirci-Delale et al. 1998), confirming that the blood samples were obtained at an accurate time during each phase, without missing the estrogen peak.

Plasma selenium and GPx activity have been shown to fluctuate similarly during the rat estrous cycle, supporting the finding of the present study (Smith et al. 1995). Das and Chowdhury (1997) also reported changes in plasma selenium concentration throughout the menstrual cycle, however, plasma selenium level was highest during the early follicular phase, and lowest during the ovulation phase. It was equivocal, however, whether blood samples were collected during estrogen peak, because the ovulatory phase was defined based on basal body temperature changes, suggesting that the estrogen peak could have been missed. In addition Das and Chowdhury (1997) did not measure dietary selenium intake nor estrogen level in plasma.

Although there were variations in plasma selenium concentration across the menstrual cycle, the concentrations at each phase were within the reference range of 53±21 to 161±19 nanogram/milliliter for healthy adults (Versieck and Cornelis 1989). The lack of change in the RBC selenium content during the menstrual cycle suggests that the metabolism and distribution of selenium in RBC is not affected by hormonal fluctuations during the menstrual cycle. This characteristic may allow RBC selenium, known to reflect the long-term intake of dietary selenium, to serve as a constant indicator of selenium status in women throughout the menstruation cycle. Similarly, Peiker et al. (1991) observed that
RBC selenium measurements did not vary throughout pregnancy, whereas serum selenium concentration decreased significantly, also suggesting that RBC selenium content is not influenced by reproductive hormone fluctuation. A reference range for RBC selenium for this population in the US has not been available. However, our result for RBC selenium content appeared to be greater than the value for 39 healthy women reported by Pleban et al. (1987) (141±14 microgram/L) but within the range reported by Snook et al. (1983) (177-247 microgram/liter) for Ohio residents.

RBC GPx activity measured at each phase was positively associated with plasma estrogen concentrations reaching the peak values during the periovulatory phase. Massafra et al. (1998) also observed cyclic variations in RBC GPx activity during the menstrual cycle. They studied 10 women with normal menstrual cycles, collecting blood samples on alternate days and found that the highest activity of RBC GPx coincided with the periovulatory estrogen peak, supporting the hypothesis that estrogen may be related to the regulation of GPx activity. Larsen et al. (1996) did not find any phase-related fluctuation in RBC GPx activity during the menstrual cycle. However, their inability to demonstrate a fluctuation in RBC GPx may have been related to the fact that they measured the RBC GPx activity only two times a week enhancing the possibility that they may have missed the peak of estrogen and corresponding GPx activity at that specific time.
The mechanisms for the observed cyclic changes in selenium parameters during the menstrual cycle are uncertain. Estrogen may be a physiological modulator, regulating selenium concentration and GPx activity directly or indirectly. This assumption is supported by the results of studies on amenorrheic women, in which exogenous estrogen treatment restored estrogen levels and RBC GPx activity to the values of normal cycling women at the mid-follicular phase (Massafra et al. 1996, Massafra et al. 1997). In contrast, when medroxyprogesterone-acetate treatment was given only, there was no increase in RBC GPx activity observed, suggesting that the increase in GPx activity was an estrogen effect (Massafra et al. 1997). Estrogen treatment has increased RBC GPx activity without changes in malondialdehyde, an indicator of lipid peroxidation (Janero 1990), suggesting that the increased GPx activity did not result from increase in lipid peroxide. The effect of treatment with estrogen containing oral contraceptives on GPx activity has been more inconsistent and dependent on the level of dose, the duration of use and the type of estrogen administered (Massafra et al. 1993, Jendryczko et al. 1993, Behall et al. 1980, Capel et al. 1981).

Estrogen's influence on the cyclic changes in selenium status appear to involve the redistribution of selenium. Roy and Liehr (1989) observed that estrogen treatment resulted in increased renal GPx activity but decreased hepatic GPx activity in male rats after 4 months of estrogen treatment whereas estrogen treatment resulted in decrease in hepatic GPx activity. These reciprocal changes in renal and hepatic GPx activity suggest that estrogen has
differential actions in these organs. Ohwada et al. (1996) also showed that uterine GPx activity was significantly elevated by exogenous estrogen treatment in spayed rats. Dietary selenium intake is known to regulate synthesis and distribution of the selenoproteins in the body. Readministration of selenium to selenium depleted rats has long been known to change selenium's distribution depending on the selenium requirement of the specific tissues (Behne and Höfer-Bosse 1984). More recently, Burk et al (1991) reported that mRNA for GPx decreased prior to that of selenoprotein P in liver and that selenium supplementation increased selenoprotein P more rapidly than GPx activity in selenium deficient rats. Although the mechanism, by which estrogen fluctuation influences the regulation of selenium status through menstrual cycle is not clear, one suggestion is that estrogen affects the maturation of bone marrow to stimulate the synthesis of active GPx (Massafra et al. 1997). Since estrogen’s effect on selenoproteins, including GPx, may be evident only after a time lag, serial blood collections even after the estrogen peak would help to determine whether estrogen's effect is through the stimulation of GPx synthesis. Although estrogen fluctuations may be affecting the distribution of selenium in different tissues, and regulating individual selenoproteins in different ways, this remains to be clarified.

It is well documented that estrogen plays a protective role against cardiovascular disease, partly by altering the lipoprotein profile in the blood (Lobo 1990) and by decreasing the likelihood of LDL peroxidation (Yagi and Komura. 1986, Mueck et al 1998). Estrogen has been considered as an antioxidant due
to the effect of its phenol-hydroxyl ring structure (Sugioka et al. 1987), in preventing lipid peroxidation (Tang et al. 1996, Ayres et al. 1998). Our results and those of other investigations suggest that the antioxidant property of estrogen may also be mediated by the modulation of GPx activity. This possible effect of estrogen on GPx activity has implications for postmenopausal women whose prolonged loss of estrogen can increase lipid peroxidation and the risk of CVD. Our findings suggest that, in postmenopausal women, a decrease in estrogen level may be accompanied by a reduction in selenium status, which may accelerate the oxidative damage menopause. It is well documented that aging is associated with increased lipid peroxidation (Schafer and Thorling 1987), which is one of the major risk factors of atherosclerosis, and that maintaining optimum antioxidant status in the elderly is important to decrease the risk of CVD. Selenium plays a role as a functional part of GPx, which protects cell membrane lipids against peroxidation during aging process (Reddy et al 1981) and low blood selenium concentrations are associated with increased risk of CVD (Salonen et al. 1982, Salonen et al. 1984, Willet et al. 1983). Therefore, a decrease in selenium status due to a decrease in estrogen in postmenopausal women may accelerate the CVD risk.

The increased activity of GPx in blood during the estrogen peak of the menstrual cycle may also be a response to elevated oxidative stress. Since estrogen is a steroid hormone derived from cholesterol, and since estrogen is known to stimulate cell proliferation and DNA synthesis (Quarmby et al. 1984,
Persico et al. 1990), during which lipid peroxide production may increase, there is possibility that lipid peroxidation is elevated during the high estrogenic phase. Whether the fluctuations in selenium and GPx activity indicate a true increase in oxidative stress or a hormone-dependent homeostatic response to oxidative stress remains.

Intake of selenium is a primary determinant for GPx activity and selenium level in blood (Levander et al. 1983). Although there have been interests for variations in food intake throughout the menstrual cycle (Pliner and Fleming 1983, Tangney et al. 1991), selenium intake during the menstrual cycle in cycling women has not been reported. Subjects in the study had dietary intakes of selenium at each phase that exceeded the recommended dietary allowance (National Research Council, 1989), of 55 microgram/day, and there were no difference observed in dietary intake of selenium across the menstrual cycle. These results not only indicated that selenium intake does not vary during the menstrual cycle but also minimized possible impact of dietary selenium on the observed fluctuations of selenium measurements during the cycle. In addition, RDA for women has been extrapolated from studies of males only. The results of this study suggest that recommendations for selenium intakes in women should consider the physiological characteristics which are distinct from men. An implication of the results of this study is that menstrual phase should be considered during the assessment of selenium status of premenopausal women in the research or clinical setting. Ignoring this factor can lead to methodological error, and misinterpretation of the result.
In conclusion, the cyclic physiological variation in circulating estrogen level influences blood parameters of selenium status in premenopausal women. These results also indicate that phase of the menstrual cycle should be considered when selenium status is estimated in cycling young women or when comparisons are made for selenium status in more than two groups who might have different hormonal profiles. The positive relationship observed between estrogen and selenium status also has implications for the population of postmenopausal women. If a decrease in selenium status is associated with the decrease in estrogen after menopause, postmenopausal women may be at increased risk of CVD development partially due to the loss of estrogen’s antioxidant defense ability possibly mediated through selenoproteins including GPx.
INTRODUCTION

Due to improvements in socioeconomic status and medical developments, human life expectancy has increased. As a result of the increase in the numbers of aged people, concerns have been raised regarding age-related health problems including cardiovascular disease, diabetes and cancer. Specifically, the increased life expectancy has resulted in women having extended postmenopausal periods bringing an attention to health problems unique to the postmenopausal years, such as the increased incidence of cardiovascular disease (CVD). CVD becomes the major cause of death for elderly women after their menopause (Henderson et al. 1986), which may be related to the estrogen withdrawal after menopause.

Numerous studies have shown that aging is associated with increased lipid peroxidation, resulting from a decrease in antioxidant enzyme status
(Schafer and Thorling 1987), which may lead to damage to lipid, protein, and DNA. Since lipid peroxides have been recognized as one of the major risk factors of atherosclerosis, maintaining optimum antioxidant status in the elderly is important to decrease the risk of CVD.

Selenium is an integral part of the antioxidant enzyme, GPx, which protects cell membrane lipid against peroxidation during aging process (Reddy et al 1981). GPx is known to decrease malondialdehyde, a byproduct of lipid peroxidation which is known to increase in disease states associated with peroxidative changes (Esterbauer 1982). Various studies provide evidence that low blood selenium concentrations are associated with increased risk of cardiovascular disease (Salonen et al. 1982, Salonen et al. 1984, Willet et al. 1983). A decrease in selenium status in postmenopausal women may accelerate the process of aging and increase their CVD risk in combination with an estrogen deficit. In spite of the importance of selenium nutrition in postmenopausal women, there have been few investigations specifically of selenium status of postmenopausal women and most of the studies have been limited to the general elderly population. In addition, studies of selenium status on the elderly have provided contradictory results, showing a decrease (Sampson 1987, McAdam et al. 1984), or no changes (Cals et al. 1997) with advancing age. These results suggest that additional factors besides age may be involved in determining selenium status in the elderly.
The proposed reasons for decreased selenium status in the elderly are low dietary intakes (Simakkala et al. 1984) especially of protein and selenium containing food (Bingham et al. 1981, Bunker et al. 1988), impaired absorption or increased excretion of selenium (Lloyd et al. 1983). A few studies have suggested a relationship between estrogen and selenium status. Selenium status has been shown to fluctuate during the menstrual cycle in female (McAdam et al. 1994) subjects, and blood selenium concentrations have been shown to fluctuate during the rat estrus cycle (Smith et al. 1995). In a study of three generations of women, Chang et al. (1994) demonstrated that selenium status was lowest in grandmothers, who had lower estrogen levels compared to their daughters and granddaughters. The results of our previous study suggest that selenium status is influenced by the fluctuation of the female sex hormone, estrogen, suggesting that changes in selenium status with age in postmenopausal women might be associated with their low estrogen levels.

The research hypothesis for the study was that the selenium status of postmenopausal women would be lower than that of premenopausal women due to a decrease in selenium intake and circulating estrogen concentration. The results of this study will further our understanding of the effect of age on the selenium status of women. This study minimized the possible effect of estrogen on selenium concentrations by collecting blood samples from premenopausal women at a phase (early follicular) in which estrogen level is as low as that of
postmenopausal women. The effect of differences in dietary selenium intake was also minimized by studying subjects with similar usual selenium intake, initially estimated using food frequency questionnaires.
METHODOLOGY

Subjects

The subjects enrolled in this study were recruited through advertisement in newspapers and fliers (Appendix M) posted on The Ohio State University campus as a part of a larger study of arachidonic acid metabolism and platelet aggregation in premenopausal and postmenopausal women with or without non-insulin dependent diabetes (NIDDM). Only the healthy non-diabetic pre- and postmenopausal control subjects were included in this study. Thirteen healthy premenopausal females aged 21 and 43 years and 10 healthy postmenopausal females aged 57 and 86 years fulfilled the study criteria. The inclusion criteria for all subjects were the following: nonsmoking, non-vegetarian, body mass index less than 30, normotensive, not on estrogen replacement therapy, nonpregnant, not taking oral contraceptives, and maintaining a regular menstrual cycle if premenopausal. Procedures for this study were approved by the Institutional Review Board of The Ohio State University, Columbus, Ohio and informed consent was obtained from each subject before enrollment in the study (see Appendix N).

Sample Collection

Two visits were made for each subject during the study period. During the first visit the subjects completed a medical information form (Appendix, O).
that included histories of medications and disease conditions and a food frequency questionnaire that was used to estimate usual selenium intake. Subjects also received instruction on how to fill out a three-day dietary records (Appendix P) prior to the second visit. Height and weight were measured and BMI was calculated and recorded. Blood pressure was measured using a sphygmomanometer on the right arm after the subject remained seated for at least 10 minutes. Subjects were required to abstain from aspirin, aspirin-based or anti-inflammatory drugs, for at least two-weeks before the second visit. For the collection of blood sample and the three-day dietary records, postmenopausal women returned within a week after the first visit. The second visit for premenopausal women coincided with the second or third day of their menses during their menstrual cycle, a time when the estrogen level in blood would be at the lowest point for all subjects. Blood was collected following a 12-hour fast, by venipuncture into vacutainer tubes containing either tri-sodium citrate (Becton Dickinson, Rutherford, NJ) or containing no anticoagulant. For both groups blood samples were separated into plasma or serum and RBC by refrigerated centrifugation. Plasma was used for analysis of selenium and GPx activity, and 17-β estradiol levels were measured in serum. Samples were stored at -20°C until subsequent analysis.

**Dietary Information**

For estimation of recent dietary intake, subjects completed a three-day diet records on the two weekdays and one weekend day before the blood
collection day (second visit). One or two days prior to beginning their dietary records, reminder calls were made to ensure the successful completion of the records. The dietary records were analyzed for energy and selected nutrients including protein, fat, carbohydrate, selenium, and vitamin E using the Food Processor program (enhanced version 7.12, 1998; ESHA Research, Salem, OR).

**Laboratory Analyses**

**Selenium levels:** Selenium concentration of plasma and red blood cells was measured using a spectrofluorometer by the modified method of Koh and Benson (1982). The reagents used were analytical grade.

The operating conditions for the spectrofluorometer were as follows: an excitation wavelength of 364 nanometers and an emission wavelength of 525 nanometers. Selenium content in the samples was manually calculated using the values from sodium selenite standards. The procedure for selenium assay is summarized in Appendix L

**Glutathione peroxidase activity:** The GPx activity of plasma and red blood cell was determined using hydrogen peroxide by the method of Paglia and Valentine (1967) (Appendix E). GPx activity was calculated based on the decrease in absorbance at 340 nanometers that was associated with the disappearance of NADPH per minute and expressed in micromoles of NADPH/gram of protein for plasma samples and micromoles of NADPH/gram of hemoglobin for RBC samples. Protein concentration in serum was assayed by
the Lowry method (Lowry et al. 1951) based on the colorimetric biuret method and measured in a spectrometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) at 500 nanometers. Hemoglobin concentration of RBC was determined spectrometrically at 540 nanometers (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) using Drabkin’s reagent. Detailed descriptions for the procedure for these assays are provided in Appendix F.

**Endocrine Test:** Concentration of 17β-estradiol in serum was analyzed using a $^{125}$I labeled radioimmunoassay kit (ICN, Costa Mesa, CA). The activity of $^{125}$I was counted using a 1470 Wizard ® automatic γ-counter (Wallac, Inc., Gaithersburg, MD). Concentration of serum 17β-estradiol was then calculated from the formula provided in Appendix Q.

**Data Analysis**

Data was analyzed using the Statistical Analysis Software (SAS) program (Release 6.12, 1988. SAS Institute Inc. Cary, NC). The Student t-test was used to detect the differences in subject characteristics, plasma selenium, plasma GPx activity, RBC selenium and RBC GPx activity between premenopausal and postmenopausal group. Pearson’s Correlations were utilized to detect any relationship between certain variables including selenium intake, selenium concentration and GPx activity in blood. Correlations were considered to be significant at $p<0.05$. All data was presented as mean ± standard error of the mean (SEM).
RESULTS

Age, anthropometric and blood pressure information for premenopausal and postmenopausal women is presented in Table 5.1. Both diastolic and systolic blood pressure were greater (p<0.05 and p<0.001, respectively) in postmenopausal women than in premenopausal women. There was no significant difference in height, weight, and BMI between the two groups. Both groups of women had mean BMI in the normal range (BMI<27).

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal Women (n=13)</th>
<th>Postmenopausal Women (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>35.8±2.0*</td>
<td>70.9±3.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162±1</td>
<td>158±2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.3±4.5</td>
<td>65.0±6.2</td>
</tr>
<tr>
<td>BMI (Kg/m^2)</td>
<td>24.3±1.1</td>
<td>26.1±1.1</td>
</tr>
<tr>
<td>DP (mm-Hg)</td>
<td>73.5±2.5**</td>
<td>80.9±2.1</td>
</tr>
<tr>
<td>SP (mm-Hg)</td>
<td>112±3***</td>
<td>138±4</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM.
Abbreviations: BMI; Body Mass Index, DP; diastolic blood pressure, SD; systolic blood pressure.
* significantly different from postmenopausal women: p<0.0001;
** significantly different from postmenopausal women: p<0.04;
*** significantly different from postmenopausal women: p<0.0001.

Table 5.1: Age, anthropometric and blood pressure data for premenopausal and postmenopausal women.
In Table 5.2, the results of 17-β estrogen, plasma selenium, plasma GPx activity, RBC selenium, and RBC GPx activity analyses are shown. Serum estradiol concentrations were not significantly different between groups and ranged from 30-118 picogram/milliliter for premenopausal women and 40-74 picogram/milliliter for postmenopausal women. Neither plasma nor RBC selenium concentrations were significantly different between premenopausal and postmenopausal women. In contrast, significantly greater plasma (p<0.02), and RBC (p<0.05) GPx activities were observed in postmenopausal women compared to premenopausal women.

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal Women</th>
<th>Postmenopausal Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-β estradiol (pg/mL)</td>
<td>70.4±8.2 (n=13)</td>
<td>54.2±4.0 (n=10)</td>
</tr>
<tr>
<td>Plasma Se (ng/mL)</td>
<td>105±125 (n=13)</td>
<td>99±26 (n=9)</td>
</tr>
<tr>
<td>Plasma GPx (U/g protein)</td>
<td>3.94±0.93* (n=13)</td>
<td>5.25±1.35 (n=9)</td>
</tr>
<tr>
<td>RBC Se (ng/mL)</td>
<td>188±36 (n=13)</td>
<td>169±46 (n=10)</td>
</tr>
<tr>
<td>RBC GPx (U/g hgb)</td>
<td>16.4±3.1** (n=13)</td>
<td>21.9±7.7 (n=10)</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SEM.

* significantly different from postmenopausal group: p<0.02;
** significantly different from postmenopausal group: p<0.05;

Table 5.2: 17-β estrogen and selenium status measures in premenopausal and postmenopausal women.
The results of the dietary analysis for intake of energy, macronutrients, selenium and vitamin E are presented in Table 5.3. No significant differences were observed between the two groups for energy, carbohydrate, protein, vitamin E or selenium intakes.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Premenopausal Women (n=13)</th>
<th>Postmenopausal Women (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie (Kcal)</td>
<td>1615±128</td>
<td>1634±111</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>199±16</td>
<td>219±17</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>63.9±5.0</td>
<td>68.1±5.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>66.6±10.6</td>
<td>58.1±7.3</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>9.34±2.12</td>
<td>9.57±1.68</td>
</tr>
<tr>
<td>Selenium (µg)</td>
<td>90.0±7.5</td>
<td>87.8±7.9</td>
</tr>
</tbody>
</table>

All values are expressed as the mean±SEM.

Table 5.3: Dietary estimates for daily intake of selected nutrients for premenopausal and postmenopausal women.
DISCUSSION

This study was designed to minimize differences in serum estradiol concentrations between premenopausal and postmenopausal women. Blood samples from premenopausal women were collected in the early follicular phase when estrogen level is at its lowest point and therefore close to that of postmenopausal women. The results indicate that there were no differences in serum estradiol between groups, eliminating the possibility that variations in estrogen concentration would affect comparisons of selenium status between the two age groups. The serum estrogen concentrations for premenopausal women were in the reference range of estrogen for the follicular phase of the menstrual cycle (30-100 picograms/milliliter) (Speroff et al. 1989a). The estrogen level for the postmenopausal women was slightly greater than the reference value of 10-20 picograms/milliliter (Speroff et al. 1989b), or the value of 26.6 picograms/milliliter reported for postmenopausal women not on hormonal replacement therapy (Predanic et al. 1998).

Measurements of selenium and GPx activity in premenopausal and postmenopausal women were in the normal range revealing that the selenium status of both groups was adequate. Plasma selenium values for women from both groups were within the range provided by several other researchers for healthy subjects. Although there have not been fixed reference values for selenium concentration in plasma or serum, a reference range of 53±21 to 161±19 nanograms/milliliter has been derived from 27 investigations in various
countries (Versieck and Comelis 1989). Age has been reported to affect serum or plasma selenium concentrations (Kallistratos et al. 1985, Sampson 1987, Simonoff et al. 1988) but the data is equivocal. Most studies have shown that serum selenium concentration increases (McAdam et al. 1984, Dickson and Tomlinson 1967) up to the age of 60 years and decreases thereafter (Sampson 1987, McAdam et al. 1984). Our results indicate that the level of plasma selenium in postmenopausal women was not significantly different from that of premenopausal women. Although low estrogen levels have been associated with decreased blood levels of selenium, postmenopausal women studied were maintaining adequate plasma selenium levels similar to those of premenopausal women during the low-estrogen phase of the menstrual cycle. This result agrees with those of Cals et al. (1997) who found that the serum selenium concentration of healthy elderly women (median=90, range=67-120) fell in the reference range for younger adults. Véronique et al. (1997) also reported that plasma selenium concentrations were similar in elderly women aged 64-82 years and younger women aged 31-40 years. In contrast, several studies have reported that plasma selenium decreases with advancing age. In most of those studies, however, institutionalized elderly were used as subjects (Ducros, 1997), dietary information was not provided (Bortoli et al. 1981), and/or men and women were grouped together (Campbell et al. 1989). It is not clear, therefore, whether reported changes in plasma selenium levels were due to age, altered dietary intake, or differences in life style and gender. It has been observed that institutionalized elderly women have reduced levels of plasma selenium
compared to those of healthy free-living elderly women (Lowik et al. 1992) often with same dietary selenium intake. In addition, elderly men have been shown to have lower selenium status than that of elderly women (Cals et al. 1997), suggesting that life style and gender also affect selenium status.

A lower selenium status has been reported in postmenopausal women (Schafer and Thorling et al. 1987) and has been associated with a reduction in food intake (Munro 1986, Health and Welfare 1977), including selenium rich foods such as meat, fish, poultry, egg, and dairy products (Bunker et al. 1988, Campbell et al. 1989). In the present study, the selenium and protein intakes of both groups were nearly identical and greater than the Recommendation Dietary Allowance (RDA) of 55 microgram/day and 50 gram/day for adult women (National Research Council 1989), respectively. In neither group, was there a linear relationship found between plasma selenium and dietary selenium level as has been reported by other researchers (Lane et al. 1983). The elderly women in the present study had greater selenium intakes than those of healthy elderly women in the UK (Bunker et al 1988) and Canada (Gibson et al 1985). Selenium intakes of only one of the premenopausal (47.53 microgram/day) and postmenopausal (54.04 microgram/day) women were slightly below the RDA. Since the diets eaten by both groups were comparable with respect to total energy, protein, fat and carbohydrate densities, it was assumed that both groups consumed diets with similar selenium density.

RBC selenium concentrations, known to reflect long-term selenium status (Neve et al. 1985), were comparable in the premenopausal and postmenopausal
groups. This result indicates that the usual dietary selenium intake of the postmenopausal women had been sufficient to maintain adequate RBC selenium concentration. There have not been enough results accumulated for RBC selenium to provide reference values. There have been no reported RBC selenium values for the postmenopausal women population in the US. Pleban et al. (1987) reported that the mean RBC selenium concentration of 39 healthy women was 141±14.3 microgram/L and Snook et al. (1983) reported a range of 177-247 microgram/liter for RBC selenium in Ohio residents. When compared to those results, both pre- and postmenopausal women in the present study were in adequate selenium status.

In contrast, the results of this study indicate that GPx activity in both plasma and RBC of postmenopausal women are greater than those of premenopausal women. Gurdol et al (1997) reported that GPx in whole blood decreased in postmenopausal women whereas Cals et al. (1997) indicated that there was no difference in RBC GPx between elderly and younger women. These differences among observations are not surprising, however, since it is well known that GPx activity varies with numerous factors such as smoking (Volkovova et al. 1996), age (Andersen 1997), gender (Guemouri et al. 1991), and diet. Although estrogen could be a factor responsible for changes in GPx activity after menopause, estrogen concentration was at a low and similar level in the postmenopausal and premenopausal women in this study. Therefore, it appears that there are other factors modulating GPx activity, resulting in an increase in the postmenopausal population of women. Perona et al. (1978)
suggested that activated oxygen increases GPx activity by showing that the addition of acetyl-phenyl-hydrazine, known to increase intracellular superoxide concentration (Carrel and Winterbourn 1974), elevated GPx activity in vitro. In clinical investigations, blood GPx activity has increased in disease states in which lipid peroxide production is increased. Uotila et al. (1993) reported that plasma and RBC GPx were elevated with increased lipid peroxide production during hypertensive complications of pregnancy. Similar patterns also have been shown in psoriasis (Corrocher et al. 1989), and diabetes (Matkovic et al. 1982, Cser et al. 1993). In each case, selenium levels varied showing either no difference, an increase, or a decrease compared to the values of controls, suggesting GPx activity increased independently from the selenium level. Results from this study also suggest that the rise in GPx activity without a change in selenium level can be explained by an adaptive response to an increased peroxide load accompanied with age. Further studies are needed to clarify the relationship between increased GPx activity and lipid peroxidation production in elderly women.

The results of the present study indicate that the healthy postmenopausal women were in good selenium status and preserved their antioxidant capability, which was comparable with that of premenopausal women. These observations may stem from the fact that our elderly subjects were in apparent good health. More attention should be given to the selenium nutrition of postmenopausal women who are at risk of low selenium status, including those who are institutionalized, have reduced selenium intake, and/or have chronic diseases in
which lipid peroxidation takes place. If selenium status is not maintained at adequate levels in those elderly women, the ability to counteract increased oxidative stress will decrease. Estrogen itself has an antioxidant property and has a protective effect against CVD development. Several lines of evidence suggest that estrogen modulates selenium status in premenopausal women, and may exert an antioxidant property that in turn reduces the incidence of CVD during a woman's reproductive years. After menopause, women are exposed to greater risk of heart disease, which may be exacerbated by the loss of the beneficial effect of estrogen modulating selenium concentration and GPx activity. The relation between lack of estrogen and its effect on selenium status in postmenopausal women remains for future investigation.

In summary, plasma and RBC selenium concentrations were similar in both groups of women despite differences in age and stage of the life cycle. Selenium status was within the normal range for both groups. Plasma and RBC GPx activity was elevated in postmenopausal women suggesting a response to oxidative processes associated with aging. Therefore, it appears important to maintain adequate selenium status in postmenopausal women in order to maintain the functional role in the antioxidant enzyme, GPx which enables a response to the oxidative stress of aging.
SUMMARY AND CONCLUSION

Throughout the life cycle a woman's body undergoes physiological changes related to alterations in hormonal profiles, which is distinct from that of males. The selenium RDA was established in 1989, however, it was based on studies using male adults only as subjects, from which RDAs for the different age and gender groups were extrapolated. This study was designed in order to examine the possible effect of the female life cycle on dietary selenium intakes and the selenium status in females at different life stages: early puberty, the menstrual cycle, and postmenopause.

The results of this study indicated the following:

1) Healthy females at different stages of life cycle consume adequate dietary selenium and maintain optimal selenium status in spite of the differences in age and stage of the life cycle;

2) Young females at early puberty consume more than double the selenium RDA for that age group, which is reflected in optimal selenium status that correlates with growth parameters;
3) During the menstrual cycle, measures of selenium status in healthy women are within the normal range, but except for RBC selenium, fluctuate along with estrogen concentration, suggesting that these changes are probably brought about under the influence of cyclic variations of estrogen but without clinical implications;

4) Healthy postmenopausal women have similar plasma and RBC selenium concentrations as those of younger women. Plasma and RBC GPx activity, however, is increased in postmenopausal women, which might be a response to age-related increase oxidative processes.

The results of this study for cycling women have implications for measurement of selenium status. The failure to consider this potential component of within-individual variability may increase the total unexplained errors of measurement, which in turn decrease the statistical power of the study, and lead to the misinterpretation of data. Because the elderly subjects in this study were apparently healthy and maintained optimal selenium concentration, the results from this study may not be applicable for postmenopausal women who are at risk for low selenium status. Therefore, special attention may be required for the older population who are at risk of low selenium status resulting from poor selenium intake, impaired absorption, and chronic diseases which increase the lipid peroxide production. If dietary selenium is not sufficient to maintain an adequate selenium status, the ability to counteract with increased oxidative stress will decrease, which may accelerate the aging process. At the same time, it should be noted that estrogen levels in the elderly women in this
study were similar to that of the premenopausal women. If postmenopausal women with lower estrogen level are studied, the result could be different. For better understanding of the relationship between estrogen and the cyclic changes in selenium status, treatment of postmenopausal or amenorrheic women with estrogen replacement to study its effect on selenium status will be another step beyond this study.

One of limitations of this study was that estradiol level was not measured in the early pubertal girls, which would help understand the possible effect of estrogen on selenium status at that stage of life. For future study of changes in selenium status during the menstrual cycle, it is suggested that serial collection of samples even after the LH surge, be measured to see if changes in selenium status continue after the sharp decrease in estrogen level. Since the menstrual cycle is regulated by various hormones interacting with each other, the change in other sexual hormones needs to be considered for the future research. In addition, the direct measurement of lipid peroxide levels in postmenopausal women would help understand to see whether the increase in GPx activity results from true increases in lipid peroxidation.

In conclusion, this study confirms that dietary selenium is a major determinant of selenium status, by showing that adequate selenium status is maintained with sufficient selenium intake throughout the female life cycle. However, the result from elderly women with increased GPx independent of selenium intake and blood selenium level suggests that another factor is associated with this parameter in these women. These results suggest that, at
each stage of the life cycle, there might be different factors differentially regulating selenium status, for example, hormone concentration during pregnancy and the menstrual cycle, and lipid peroxidation after menopause and in certain diseases.
REFERENCES


Burk RF, Gregory PE. Some characteristics of $^{75}$Se-P, a selenoprotein found in rat liver and plasma, and comparison of it with selenoglutathione peroxidase. Arch. Biochem. Biophys. 1982; 212:73-80.


Calvin HI, Cooper GW, Wallace EW. Evidence that selenium in rat sperm is associated with a cysteine-rich structural protein of the mitochondrial capsule. Gamete. Res. 1981a; 4:139-49.


Channing CP, Anderson LD, Hoover DJ, Kolena J, Osteen KG, Pomerantz, Tanabe K. The role of nonsteroidal regulators in control of oocyte and follicular maturation. Rec Prog Horm Res 1982;38:331-408


160


Gey KF. In the antioxidant hypothesis with regard to arteriosclerosis. Bibliotheca Nutrio et Dieta. 1992;37:53-91.


Hill KE, Lloyd RS, Burk RF. Conserved nucleotide sequences in the open reading frame and 3' untranslated region of selenoprotein P mRNA. Proc Natl Acad Sci USA. 1993;90:537-41.


Hutchison JS, Zeleznik AJ. The rhesus monkey corpus luteum is dependent on pituitary gonadotropin secretion throughout the luteal phase of the menstrual cycle. Endocrinology 1984;115:1780-6.


Li JR. Investigation on the relationship between environmental selenium level and the incidence rate of KBD. Chin J Endemiol. 1989;8:129-34.


175

Meinhold H, Haselbach U, Walzog B, Behne D. Thyroid hormone synthesis, peripheral deiodination and kinetics in longterm selenium deficient rats. Thyroid 1991;Suppl 1.:S-23 (Abs)


Milman N, Hanse JC, Mathiassen B, Bohm J. Serum levels of bromine, copper, zinc and selenium in a Greenlandic Inuit hunter population from the Thule district. Trace Elem Med 1993;10:60-5.


Quarmby VE, Korach KS. The influence of 17 beta-estradiol on patterns of cell division in the uterus. Endocrinology 1984;114: 694-702


189


Ursini F, Maiorino M, Valente M, Ferri L, Gregolin C. Purification from pig liver of a protein which protects liposomes and biomembranes from peroxidative degradation and exhibits glutathione peroxidase activity on phosphatidylcholine hydroperoxides. Biochim Biophys Acta. 1982;710:197-211.


Ursini F, Bindoli A. The role of selenium peroxidases in the protection against oxidative damage of membranes. Chem Phys Lipids. 1987;44:255-76.


Wallace E, Calvin HI, Cooper GW. Progressive defects observed in mouse sperm during the course of three generation of selenium deficiency. Gamete Res. 1983; 4:377-87.


Zeleznik AJ. Premature elevation of systemic estradiol reduces serum levels of follicle-stimulating hormone and lengthens the follicular phase of the menstrual cycle in rhesus monkeys. Endocrinology. 1981;109:352-5


APPENDIX A, CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE FOR FEMALES DURING EARLY PUBERTY
CONSENT TO SPECIAL TREATMENT OR PROCEDURE

You are asked to participate in a clinical research program to evaluate relationship between diet and skeletal formation. We think that the best protection against osteoporosis ("brittle bone disease") later in life is to have strong bones when the skeleton reaches maturity (adulthood). We believe that the critical time for building strong bone is during early adolescence (early teenage period) when needs for skeletal minerals are the highest.

I, _____________________, hereby authorize or direct V. Matkovich, M.D., Ph.D. or associates of his choosing, to perform the following treatment: (described in general terms)

Prior to inclusion into the study I will give detailed information about my dietary habits including which type, how often and how much food I eat. I will also complete the self reported form (Tanner's rating) about my physical development (secondary sexual characteristics-breasts and pubic hair). If information from both forms meet the inclusion criteria of the study, I will be selected to participate in the study which will last 4 years.

In that case, I will continue to give the detailed information of my dietary habits, physical development, as well as of my activity level, every 6 months. Blood pressure, height and weight will be measured every six months during the four-year period, together with bone mass density at forearm and skeleton (whole body). Body fat will be measured twice a year, by the bioelectrical impedance method and dual energy X-ray absorptiometry.

I will also have X-ray of my hand taken at the beginning and at the end of the study (2 times in total), for the purpose of determining the skeletal age. A 24-hr urine collection and approximately 2 tea-spoons of blood drawn from the arm vein will be taken at the beginning of the study, and every 12 months.

I will be taking four supplemental tablets daily throughout the 4-year study period. I will not know which tablet I am taking: the one with or without calcium.

Biological parents of participating girls will be asked to participate in the bone mass measurement, only once during the whole study.
All tests and measurements will be done at The Ohio State University. To complete a dietary intake and activity level forms takes 30 minutes. The measurements which will be done on the site are as follows: blood pressure, height and weight measurements; 15 minutes, hand X-ray 10 minutes; bioelectrical impedance 5 minutes; forearm densitometry 15 minutes, and the whole body bone, fat and soft tissue measurements about 20 minutes. It takes about 1 hour of time for each visit, or 9 hours during the 4 year study period. There will be a total number of 300 participants. 24 participants will be asked (not required) to take part in a 2 week in-patient metabolic study at the University Hospital.

In addition to the above I realize that I could participate in a study entitled "Nutrition & Human Growth – Enhancing Middle School Science", in my school. I will be analyzing data collected on myself and/or on my colleagues under the supervision of my science teacher. The data collected on others will be coded so that I will not know the names of the participants. The purpose of this is to stimulate science education in the school and therefore will help my directly.

Upon _______MYSELF________.

The experimental (research) portion of the treatment of procedure is:

I, along with other participants who will be selected for the study, will take by mouth four tablets per day (two in the morning and two during the evening) throughout the 4-year period. One half of the participants will be given placebo (tablet without calcium) and the other half will be taking. This, as I understand, is the rule of a double blind clinical trial in which I am participating. I will have my dietary interview, physical examination with anthropometry (height & weight measurements), and bone mass measurements at forearm and the whole body by non-invasive techniques done at baseline (beginning of the study) every 6 months over 4 years. Hand X-rays will be taken at the beginning of the study and every 12 months. I will collect five 24-hour urine samples throughout the 4-year study; every 12 months.

This is done as part of an investigation entitled:

Influence of Calcium on Bone Mass Formation During Puberty

1. Purpose of the procedure of treatment:

To find adequate prevention for osteoporosis (brittle one disease) with dietary manipulation during puberty period.
Protocol No. 90H0002

2. Possible appropriate alternative methods of treatment.
   **Not to participate.**

3. Discomforts and risks reasonably to be expected:

The risks of taking part in this study are minimal. The placebo tablets of the supplemental calcium tablets, has no harmful effect on human health. A high mineral diet certainly is contraindicated in patients with kidney stone disease, because it can further increase the risk of forming stones. Mineral tablets can sometimes cause mild constipation. Subjects with bone or kidney stone will not be included in the study. All participants will continue with their regular diets throughout the study period.

I understand that the placement of a needle in the arm vein to draw blood may cause slight discomfort and a bruise may form at the site of needle puncture as well as fainting. The total amount of blood drawn during the study will be about 50 cc (1.5 oz). The only possible invasion of my privacy will be done during the dietary interviews and determination of sexual development (Tanner staging).

I will be exposed to the external irradiation of about 0.3x2 mRads annually, from whole body bone mass measurements and 0.1x2 mRads from forearm measurements. This is total of 0.8 mRads annually, or 0.27% of annual natural exposure. In addition, at the beginning and end of the study I will have my hand X-ray taken, and I will be exposed to 10 mRads of irradiation each time to the hand only, which is amounts to 3.3 of the total natural dose for one year. I realize that this is a minimal irradiation and far below the natural annual irradiation exposure from environment which is 300 mRad. It is equal to the irradiation received on the airplane during a single 20 min. flight, or skiing in the high mountains over the period of 1-3 days.

4. Possible benefits for subjects/society:

There will be several benefits to me, the participant: 1) physical examination, 2) dietary evaluation, 3) urine and blood tests, and 4) bone mass measurements. Society may benefit by the finding of a better prevention for osteoporosis by simple dietary manipulations. I will receive compensation after each evaluation ($30). There will be total of 9 evaluations for which I will receive $270. If I remain in the study till the end of the four year period, I will receive a special bonus. I understand that I might be asked to participate in an in-patient metabolic study for which I will receive extra compensation.

5. Anticipated duration of subject's participation:

**I will participate in the study which will last 48 months with 9 visits to O.S.U.**
Protocol No. 90H0002

I hereby acknowledge that Dr. V. Matkovich or his collaborator has provided information about the procedure described above, about my rights as a subject and that he/she answered all questions to my satisfaction. I understand that I may contact him/her at (614) 293-3838 should I have additional questions.

I understand that where appropriate, the U.S. Food and Drug Administration may inspect my records pertaining to this study. I understand further that the records obtained during my participation may be made available to the sponsor for this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given me concerning this treatment of procedure.

In the unlikely event of physical injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of The Ohio State University. I understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: __________________________ Time: _______ A.M.

P.M.  

Subject: ______________________________________

Signed: ______________________________________

(Parent or guardian)

Witness: ______________________________________

____________________________________________

Person Authorized to consent for subject- if required

I certify that I have personally completed all blanks in this form and explained then to the subject of his/her representative before requesting the subject of him/her representative to sign it.

Signed: ______________________________________

(Signature of the Project Director of Authorized Representative)

HS-028A(Rev. 4/89)
APPENDIX B, EVALUATION FORMS FOR, PUBERTAL STAGE
Have you started your period? Yes No
Date when started: ____________________________
First day of last period: ________________________
Duration of period (days): ______________________
Average cycle (Days from one period to the next)
Number of periods per 6 months
Are you on any contraceptive pills?
Yes____ No____
or other contraceptive agent or device?
Yes____ No____
Could you be pregnant?
YES____ NO_____
Check the stage that best represents your development.

1.  
2.  
3.  
4.  
5.  
6.
MEDICAL CAMP RECORD SHEET

NAME ____________________________

Have you started your period? _____

First day of last period? _____

Duration of period? _____

Please fill out this record sheet every day. This information is very important for the study. Answer this question:

Are you having your period?

<table>
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<tr>
<th>Date</th>
<th>YES</th>
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APPENDIX D, METHODS FOR SELENIUM ANALYSIS
BY GAS CHROMATOGRAPHY
Reagents

1. Nitric acid solution:
   a. Measure 30 g of magnesium nitrate hexahydrate
   b. Add 100 mL of nitric acid.

2. Hydroxalamine sulfate, EDTA, and Urea solution:
   a. Measure 5 g of hydroxalmine sulfate, 5 g of EDTA, and 75 g of Urea.
   b. Fill up to 500 mL with distilled and deionized water.

3. Complexing solution:
   a. Measure 0.125 g of chloride salt of 4-nitro-o-phenylenediamine (4NPD).
   b. Fill up to 25 mL with 1 N HCl.
   c. Store in the amber bottle in the freezer.

4. 1 N HCl:
   a. Put 96 mL of 36% HCl
   b. Fill up to 1 liter of distilled and deionized water

Procedure

Digestion

1. Add 20-500 microliters of RBC or plasma, 50 microliters of 0.5 ppm selenium standard solution, or 20 milligrams of reference standard (bovine liver) to 7 millimeter Pyrex weighing vial.

2. Add 1.5 milliliters of nitric acid containing 30 g of magnesium nitrate hexahydrate/100 milliliters of acid.

3. Place in modular dry-bath for 60 minutes at 105 °C, followed by 30 minutes at 115 °C, and 120 minutes or more at 130 °C.

4. Remove vials from modular dry-bath. Make sure that sample look thick (almost dry).

5. Transfer the vials onto a hot plate on high setting for 1 or more hours.
6. Raise the temperature gradually. Watch carefully that no samples “spits out”. If there are bubbles rising rapidly, reduce the temperature.

7. Remove from the hot plate when fumes of nitrogen oxide ceases and the sample looks like a yellowish-white ash.

8. Place in a muffle furnace at 500 °C for 60 minutes.

9. Allow vials to cool in muffle furnace several hours (preferably overnight after muffle furnace is turned off) before opening door and removing.

**Complexing and Extraction.**

1. Add 1.5 milliliters of 36% hydrochloric acid (analytical grade for trace element assay) to cooled ash along with the wall of the vials.

2. Cap vials lightly and place in modular dry-bath at 90 °C for 15 minutes, making sure ash is completely dissolved.

3. Cool to room temperature.

4. Add 2.5 milliliters of solution of 1% hydroxalamine sulfate, 1% EDTA, and 15% urea to each vial.

5. Mix and allow to stand for 10 minutes.

6. Add 100 microliters of complexing solution and mix.

7. Place vials in modular dry-bath at 45 °C for 30 minutes.

8. Cool to room temperature.

9. Add 1 milliliter of toluene and mix for 5 seconds.

10. Remove approximately 0.5 microliters of the toluene layer (organic phase) with pipettor.

11. Place in screw cap vial with Teflon cap liner for subsequent gas chromatographic analysis.
Gas Chromatography

1. Instrument:
   Varian 3300 Gas Chromatograph with Electron Capture Detector (ECD)
   and Make-Gas attachment (Sugarland, TX).

2. Column:
   J & W Megabore (530 micron I.D.). DB 225, 15 meters, 1.0 micrometer
   Film thickness (J & W Scientific, Folsom, CA)

3. Carrier Gas: nitrogen (56-60 psi).

4. Temperature of operation:
   Column 190 °C
   Injector 220 °C
   Detector 300 °C

5. Software: Star Integrator.
APPENDIX E, ANALYTICAL METHOD FOR GLUTATHIONE PEROXIDASE ACTIVITY
GLUTATHIONE PEROXIDASE ACTIVITY ANALYSIS
FOR RED BLOOD CELLS (RBC) AND PLASMA
(Paglia and Valentine 1967)

Reagents

1. Glutathione (GSH) reductase solution:
   a. Pipette 25 microliters of GSH stock solution (1000 units/mL)
   b. Dilute to 5 mL with K buffer solution

2. Glutathione (GSH) solution (40 mM):
   a. Weigh 0.0615 g of GSH into 5 mL volumetric flask.
   b. Add 1-2 mL of distilled water.
   c. Adjust pH to 7 with 1 M KOH
   d. Fill to 5 mL with distilled water.

3. NADPH solution (20 mM):
   a. Measure 0.00833 g of NADPH into microcentrifuge tube.
   b. Dilute to 500 microliters with 0.1 M NaHCO₃
   c. Vortex well.

4. NaN₃ solution (2.5 M):
   a. Dilute 1.625 g of NaN₃ of 10 mL with distilled water.

5. H₂O₂ solution (0.011 M):
   a. Dilute 21.3 microliters of 30% H₂O₂ to 25 mL distilled water.

6. KH buffer solution:
   a. Add following chemicals in 500 mL volumetric flask.
      4.50 g NaCl
      0.23 g KCl
      1.05 g KH₂PO₄
      0.19 g MgSO₄
      1.35 g NaHCO₃
      0.45 g Glucose
   b. Fill to 500 mL with distilled water.
   c. Adjust pH to 7.40 with 1.0 M KOH.
7. K buffer solution:
   a. Add 10.875 g of K<sub>2</sub>HPO<sub>4</sub> and 2.3763 g of EDTA into 250 mL volumetric flask A and fill with distilled water.
   
   b. Add 3.40 g of KH<sub>2</sub>PO<sub>4</sub> and 0.9505 g of EDTA into 100 mL volumetric flask B and fill with distilled water.
   c. Add the content in flask B to flask A to adjust pH equals to 7.40.

Procedure

1. Set up Gilford Response UV-VIS Spectrometer (Gilford Instrument Laboratories, Oberlin, Ohio).
   b. Scan length: 5 minutes
   c. Temperature: 37°C

2. Add the following to the optically matched quartz cuvettes and incubate at 37°C for 10 minutes:
   a. 200 microliters of glutathione reductase solution
   b. 50 microliters of 40 mM glutathione solution
   c. 500 microliters of KH buffer for blank or diluted samples according to the condition below;

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount (μl)</th>
<th>KH Buffer (μl)</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>20</td>
<td>980</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>12 (50x dilution)</td>
<td>1080</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>60 (50x dilution)</td>
<td>1140</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>20 (50x dilution)</td>
<td>980</td>
<td>2500</td>
</tr>
<tr>
<td>Plasma</td>
<td>20</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1080</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>1152</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>600 (25x dilution)</td>
<td>600</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>120 (25x dilution)</td>
<td>480</td>
<td>50</td>
</tr>
</tbody>
</table>
   
   d. 210 microliters of distilled water

3. Remove the cuvettes from the water bath and add 10 microliters of 20 mM NADPH solution and mix.
4. Add 20 microliters of 2.5 M sodium azide solution, and swipe the sides of the cuvettes with kim wipe.

5. Add 10 microliters of 0.011 M hydrogen peroxide solution, and mix quickly.

6. Run on spectrophotometer and read the absorbance.
APPENDIX F, METHODS FOR TOTAL PROTEIN AND TOTAL HEMOGLOBIN ANALYSIS
MEASUREMENT OF TOTAL PROTEIN
(Lowry et al. 1951)

Reagents

1. Alkaline copper reagent
   a. diluting reagent:
      2 gram Na₂CO₃
      dilute to 100 mL with 0.10 N NaOH
   b. alkaline copper reagent:
      1000 µl 1% CuSO₄·5H₂O
      1000 µl 2% Na tartrate
      dilute to 100 mL with diluting agent

2. Folin reagent
   5000 µl phenol reagent (Folin reagent)
   5000 µl distilled water

3. Lysozyme working standard solution
   0.0125 g lysozyme stock
   dilute to 25 mL with distilled water.

Procedure

1. Label tubes in duplicate.

2. Add samples and distilled water to make 1200 microliters in tube.

3. Prepare standard in duplicated as follows;

<table>
<thead>
<tr>
<th>Tube</th>
<th>Volume of Standard (µl)</th>
<th>Volume of water (µl)</th>
<th>Protein Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>1200</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>1150</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1100</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>700</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>200</td>
<td>500</td>
</tr>
</tbody>
</table>
4. Add 6.0 milliliters of alkaline copper reagent to each tube and mix well. Let stand 10 minutes.

5. Add 300 microliters of Folin reagent to each tube and mix. Let stand 30 minutes.

6. Read absorbance in a spectrophotometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, Illinois) at 500 nm.
MEASUREMENT OF TOTAL HEMOGLOBIN

Procedure

1. Label test tubes in duplicates.

2. Add 5.0 milliliters of Drabkin’s solution to each test tube.

3. Add 10 microliters of red blood cells, rinsing pipet capillary tube 3-4 times with the reagent in the test tube to assure complete blood transfer. Vortex well.

4. Prepare working standard solutions.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Hgb* Standard</th>
<th>Drabkin’s Solution</th>
<th>Hgb* Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.0 milliliters</td>
<td>6.0 milliliters</td>
<td>0.0 g/100 milliliters</td>
</tr>
<tr>
<td>1</td>
<td>2.0 milliliters</td>
<td>4.0 milliliters</td>
<td>6.0 g/100 milliliters</td>
</tr>
<tr>
<td>2</td>
<td>4.0 milliliters</td>
<td>2.0 milliliters</td>
<td>12.0 g/100 milliliters</td>
</tr>
<tr>
<td>3</td>
<td>6.0 milliliters</td>
<td>0.0 milliliters</td>
<td>18.0 g/100 milliliters</td>
</tr>
</tbody>
</table>

*Hgb: hemoglobin

5. Set spectrophotometer (Bausch and Lomb Spectronic 20, Leica, Inc., Deerfield, IL) to 540 nm and zero with blank.

6. Measure the absorbance if each working standard at 540 nm and construct a standard curve plotting the absorbance of the standards against hemoglobin concentration.

7. Determine total hemoglobin concentration (g/100 milliliters) of unknown directly from calibration curve.
APPENDIX G, ADVERTISEMENT FOR SUBJECTS
FEMALE SUBJECTS NEEDED
FOR
NUTRITIONAL RESEARCH

Female subjects are needed for the study designed to learn how the status of blood selenium, an essential element and antioxidant in human body, changes with the estrogen fluctuation during menstrual cycle.

To be participants, the subjects should meet the following study criteria
- Healthy premenopausal women (Age between 18-38 years) with regular menstrual period
- Nonpregnant and not using oral contraceptive and/or vitamin supplement
- Nonsmoker

This study requires at least three clinic visits throughout one menstrual cycle for blood drawing and dietary data collection.

Subjects will receive $60.00 upon their successful completion of the study. Individual results including body mass index, blood pressure, and changes in blood selenium and estrogen status during menstrual cycle will also be provided.

If you meet the criteria and are interested in taking part in the study, contact:

Dr. Anne Smith
347 Campbell Hall
Dept. of Human Nutrition & Food Management
1787 Neil Ave. Columbus, OH 43210
614) 292-0715
APPENDIX H, CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE FOR FEMALES DURING THE MENSTRUAL CYCLE
CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

I,________________________, hereby authorize or direct Dr. Anne Smith, associates or assistants of his/her choosing, to perform the following treatment or procedure (describe in general terms). In this study, the subjects are supposed to be nonsmoker, nonuser of oral contraceptives or vitamin/mineral supplement at the time of enrollment and in generally good health without chronic diseases. As part of the study, I agree to be asked to do things as follows;
- I will keep a record of my food consumption for the 3 days prior to the blood sampling.
- I will use home urine analysis kit to identify ovulation.
- My height, weight and blood pressure will be measured.
- I will visit data collection site 3 times at specific period of menstrual cycle and have a blood sample drawn from vein about 20 ml (4 teaspoons or 2/5 of an ounce).

( myself or name of subject)

The experimental (research) portion of the treatment or procedure is:

In order to study the effect of estrogen on changes in selenium status, blood selenium and estrogen levels will be measured at different stages of menstrual cycle in premenopausal women. Those values from females at different stages of the reproductive cycle (puberty, pre- and post-menopause) will be compared to see if there is any change in selenium status as they age also. There is no experimental treatment involved.

This is done as part of an investigation entitled:
Does female selenium status fluctuate with estrogen level?

1. Purpose of the procedure or treatment:
The purpose of this study is to provide information about how the selenium level changes during the menstrual cycle and throughout the life cycle and to determine if the amount of selenium needed in the diet changes during a women's life cycle.

2. Possible appropriate alternative procedure or treatment (not to participate in the study is always an option):
I may choose not to participate or to withdraw at any time without loss of any benefits to which I am otherwise entitled. If I am pregnant or using oral contraceptive I am not eligible for the study. This information will be confirmed by the hormonal test done as part of the study.

3. Discomforts
There could be risks of venipuncture (taking blood from a vein) including discomfort and/or bruising at the site of the puncture. Less commonly, a small clot, swelling of the vein, or bleeding may occur at the puncture.

4. Benefits
I will receive the benefit of in-depth clinical evaluation. The overall benefit of the study is the possible improvement in the design of recommendation regarding selenium intake and assessment of selenium status for females throughout the life cycle, especially during puberty, pregnancy, and the postmenopausal years.

5. Anticipated duration of subject's participation (including number of visits):
I will be asked to come to data collection site 3 times at specific periods of menstrual cycle (total duration to complete participation would be approximately 4 weeks)
I hereby acknowledge that Dr. Anne Smith has provided information about the procedure
described above, about my rights as a subject, and he/she answered all questions to my satisfaction.
I understand that I may contact him/her at phone No. 614) 292-0715 should I have additional
questions. He/she has explained the risks described above and I understand them; he/she has also
offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records
pertaining to this study. I understand further that records obtained during my participation in this
study that may contain my name or other personal identifiers may be made available to the sponsor
of this study. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time
after notifying the project without prejudicing future care. No guarantee has been given to me
concerning this treatment of procedure.

I understand in signing this form that, beyond giving consent, I am not waiving any legal rights that I
might otherwise have, and I am not releasing the investigator, the sponsor, the institution, or its
agents from any legal liability for damages that they might otherwise have.

In the event of injury resulting from participation in this study, I also understand that immediate
medical treatment is available at University Hospitals of The Ohio State University and that the costs
of such treatment will be at my expense; financial compensation beyond that required by las is not
available. Questions about this should be directed to the Office of Research Risks Protection at 292-
5958.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been
given to me.

Date: _______________ Time ______________ PM Signed: _________________________________
(Subject)

Witness (es) _________________________________ _____________________________________
If Required ___________________________________ (Person Authorized to Consent for
Subject if Required)

I certify that I have personally completed all blanks in this form and explained them to the subject or
his/her representative before requesting the subject or his/her representative to sign it.

Date: _______________ Signed: _________________________________
(Signature of Project Director or his/her
Authorized Representative)
APPENDIX I, PRESCREENING FORM FOR PERSONAL INFORMATION
Subject ID#________  
Name __________

Last Name ___________________  First Name ___________________

Date of Birth: _______________  Marital status: _______________

Weight: _______________  Height: _______________

Occupation: _______________  Possible visiting hr: _______________

Phone#: _______________  Hrs of Exercise per wk: _______________

OCC: _______________  Suppl. or medication: _______________

Menstrual cycle information;

Length of cycle: _______________

1st day last period: _______________  Next cycle: _______________

<table>
<thead>
<tr>
<th>Expecting date</th>
<th>Actual date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Next cycle</td>
<td>_________</td>
</tr>
<tr>
<td>LH surge</td>
<td>_________</td>
</tr>
<tr>
<td>Ovulation</td>
<td>_________</td>
</tr>
<tr>
<td>ML</td>
<td>_________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Expected visiting date</th>
<th>Actual visiting date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>_________</td>
</tr>
<tr>
<td>2nd</td>
<td>_________</td>
</tr>
<tr>
<td>3rd</td>
<td>_________</td>
</tr>
<tr>
<td>4th</td>
<td>_________</td>
</tr>
<tr>
<td>5th</td>
<td>_________</td>
</tr>
</tbody>
</table>
APPENDIX J, QUESTIONNAIRES FOR MEDICAL HISTORY
AND PERSONAL INFORMATION
Demographic Information and Medical History Questionnaire

Name: ___________________________  Date: ___________________________

ADD.: ____________________________

PHONE: _______________

Date of Birth: ____________  Marital Status: __________

Ethnic: ___________  Occupation: _____________

GENERAL INFORMATION

1. How many persons live in your home (include yourself)?: __________

2. The highest grade or year of school you have attended
   Never attended: __________
   Elementary: ______________
   High School: ____________
   College: ________________
   Graduate School+: __________
   Others: ________________ (specify the type of school)

3. Have you had any weight changes recently? Yes ___  No ____
   If yes, please specify ______________________________________

4. What is your level of activities?
   Sedentary _____  Moderately active _____  Very active _____

5. Have you involved in any activity or sports? Yes _____  No _____
   If yes, please specify followings
   Type ______________________
   How often __________________
   How long __________________

6. Have you been on any type of special diet? (include diet for weight reduction)
   :  
   Yes ____  No _____
   If yes, please specify the type and duration
   __________________________________________________________
7. Do you have food allergies, or is there any foods you dislike or avoid?
   Yes ___ No ___
   If yes, please specify the item and the reason __________________________

8. What is the average household income for the last two years?
   ______________________ (Include all sources such as salaries, government assistance, food stamps, social security, etc.)

9. Income spent on Food: ______________________ per month or week
   (Circle one)

**MEDICAL and CLINICAL INFORMATION**

1. Check the following health conditions that you have experienced or diagnosed (If yes, complete all that apply and if none, please indicate)
   None ________

<table>
<thead>
<tr>
<th>Condition</th>
<th>Year of Onset</th>
<th>Brief Description (Include Familial History)</th>
<th>Still Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Vascular Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Endocrine Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.I. Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatologic Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Date of Menarche (First period) __________________

3. Average length of a menstrual cycle _________ days

227
4. The first day of last period __________

5. Do you have regular menstrual cycle?  Yes ___  No ___  
(Yes if the length of cycle did not differ more than 2 days for previous 3 month)

6. Do you have premenstrual symptoms?  Yes ___  No ___  
If yes, please check following questions

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Degree of severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal cramping</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
</tr>
<tr>
<td>Mood changes</td>
<td></td>
</tr>
<tr>
<td>Swelling</td>
<td></td>
</tr>
<tr>
<td>Changes in appetite</td>
<td></td>
</tr>
</tbody>
</table>

7. Do you take any medication to relieve the premenstrual symptoms?  
Yes ___  No ___  
If yes, please specify the name and the amount of drug ____________

8. Do you have children?  Yes _____  No ___  
If yes, how many? ________  The year of the last pregnancy ______

9. Are you breast feeding?  Yes ____  No ___

10. Have you taken oral contraceptive within last 1 year?  Yes ___  No ___

11. Have you taken any vitamin or mineral supplement within last 6 months?  
Yes ___  No ___  
If yes, please answer next questions

<table>
<thead>
<tr>
<th>Type of supplements</th>
<th>Brand</th>
<th>#of pills taken/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple vitamins without minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple vitamins plus minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Vitamin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Specify which vitamin _______)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single mineral</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
12. Please list any other medications you have taken with last 6 months.__________________________________________________________

13. Please specify if you have been on any hormonal therapy in the past.________________________________________________________

14. Do you smoke? Yes ____ No ____
If yes, how many cigarettes per day? ________________

15. Do you drink alcohol? Yes ____ No ____
If yes, how often? ________________
how many ounces at one time? ________________
Which type of alcohol? ________________

*Weight ________________  BMI ________________
Height ________________  Blood Pressure ________________
APPENDIX K. THREE- DAY DIETARY RECORDS
THREE DAY FOOD RECORD

This set of food record forms is for you to complete before your next appointment (around ____________)

Please fill out the forms several days before your next appointment and bring them with you.

Thank you very much for your corporation in this important study.
INSTRUCTIONS FOR COMPLETING THE FOOD RECORD

Choose three typical days (2 weekdays and 1 weekend day). Try to eat the way you usually do while you are keeping the food record. Please follow the instructions below as carefully as you can.

If you have any question, please call: 292-0715 or 431-3462

INSTRUCTION

1. Record all the food you eat or drink for each day you select. Most people find that it is helpful to do this as soon after the meal or snack as they can.

2. Write only 1 food item on a line.

3. Describe the type of food eaten as clearly as you can. Use the samples provided on page 5 as a guide.
   - List ingredients to help describe any unusual casserole or salad.
   - Indicate whether the food is canned, fresh, frozen, or diet.
   - List the brand names of food if you know them.

4. Describe the amounts of food you eat and drink as clearly as you can. Use the following examples as a guide.
   - **Liquid**: List as cups (cup is equal to 8 ounces), parts of cups, or fluid ounces.
   - **Meat, fish, cheese, eggs**: List in ounces, by number, or size. Specify if the amount given is in cooked or raw weight.
     
     Ex: Lean ground beef patty Broiled 1/4 lb. raw
     Cheddar cheese Kraft 1 slice (4" 3" 1/8")

   - **Fruits**: list as cups, parts of cups, or by number. Include the size (diameter and/or length) of fresh fruits.
     
     Ex: Banana 1 small (6" long)

   - **Vegetables**: list as cups, parts of cups, or by number.
     
     Ex: Green beans Delmonte 1/2 cup

   - **Bread, Rolls, Crackers**: list by number or size.
     
     Ex: Whole wheat bread Ovenjoy 1 slice
     Triscuits Nabisco 4 pieces

   - **Cereal, rice, noodles, potato**: list by cups, parts of cups, size or number.
     
     Ex: Spaghetti Cooked 1 cup
-Pancake, Waffles: list by number and size
   Ex: Waffle Betty Crocker 2 (5” diameter)

-Fats: margarine/butter-list by teaspoons, tablespoons, or pats.
   salad dressing, cream, oil, gravy-list by teaspoons, tablespoons, or cups

-Bacon, sausage: list by number of slices or links

-Sweets: list by teaspoons or tablespoons for jelly, honey, sugar and
   syrup, and by number and size of bar or pieces for candy

-Desserts: Jello, puddings, ice-cream-list by cups or parts of cups
   Cookies-list by number and size
   Pie, and cake-list by number and size (length and width at longest end)

   Ex: Cookie, choc. chip Mrs. Fields 1 (2 1/2” diameter)
       Ice-cream, cone Hagen Daz 1 scoop, sugar cone
       Choc. cake with choc icing Homemade 1/10 of 9” layer cake

5. Describe how the food was prepared. For example: baked, broiled, raw, scrambled or
   other. Include butter, margarine, oil, sauces, dressings, gravies, dessert toppings added in
   cooking or at the table.

6. Eating out:
   Give the name of the restaurant so that we may call for more information if necessary.
   Describe the food item eaten as carefully as you can.

   Ex: Pizza Hut Pizza sausage and cheese 1 slice of med. 4” 6”
       Wendy’s junior ham burger with cheese

7. Remember to list everything you eat or drink including gum, cough drops, pickles, catsup,
   tartar sauce.

8. If you take a vitamin-mineral supplement, please write down how much you take and the
   brand name.
## Food Record

**Subject ID:**

**Visit No. 1**

**Name:**

**Date:**  
(Weekday/Weekend)

**Phone:**

| Day/Time | Kind of Food | How Prepared | Brand Name | Amount or Size of Serving | For Research Use
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*Do Not Fill In*
APPENDIX L, FLUOROMETRIC METHODS FOR SELENIUM ANALYSIS
FLUOROMETRIC ANALYSIS OF SELENIUM
(Koh and Benson 1983)

Reagents

1. Disodium Ethylenediamine Tetraactate (Disodium EDTA) solution (0.1M):
   a. Measure 32.2 gram of disodium EDTA
   b. Dissolve in 1 liter of double distilled and deionized water.

2. 2,3-Diaminonaphthalene (DAN) solution (0.1%):
   a. Weigh 0.1 gram DAN into 250 mL Erhlemeyer flask.
   b. Add 100 mL 0.1M hydrochloric acid and place in 60 °C water bath.
      agitate flask occasionally to help dissolution of DAN.
   c. In hood, transfer DAN solution to separatory funnel.
   d. Add 25 mL cyclohexane per 100 mL DAN solution and shake it vigorously
      for 30 seconds.
   e. Let phases separate.
   f. Drain aqueous phase (DAN) into clean Erhlemeyer flask.
   g. Repeat washing procedure 3 more times, discarding cyclohexane layer
      each time.
   h. Filter DAN into clean Erhlemeyer flask to remove cyclohexane.
   i. Store at 4 °C until use.

3. Cyclohexane:
   Analytical reagent grade.

4. Nitric Acid:
   Analytical reagent grade.

5. Perchloric acid:
   Analytical reagent grade (70%)

6. Hydrochloric acid (0.1 M):
   Mix 9.8 mL of concentrated hydrochloric acid (37 %) with 1 liter of double
   distilled and deionized water.

7. Digestion acid:
   Mix 4 parts HNO₃ and 1 part 70% perchloric acid.

8. Selenium standard solution:
   a. Make working standard solution by dissolving 3.951 milligram of sodium
      selenite in 100 milliliters of 0.1 M hydrochloric acid.
b. In 5 separate volumetric flask, put 0.5, 1.0, 2.0, 3.0 and 4.0 milliliters of selenium working standard solution.
c. Fill up to 500 milliliters with 0.1 M hydrochloric acid.
d. Store calibration standards at room temperature in Nalgene bottles.

Procedure

Decontamination of glassware

1. Wash all the glasswares with lab soap.
2. Rinse thoroughly with hot tap water.
3. Swirl concentrated nitric acid covering entire inside walls of culture tube and any glassware.
4. Triple rinse with deionized water and let them dry

Digestion

1. Pipet 1 milliliter of sample, each concentration of calibration selenium standards, and blank (0.1 M hydrochloric acid into 25 x 200 millimeter screw cap culture tube.
2. Add boiling stones to culture tube to avoid bumping of sample while digestion.
3. Wash down perchloric acid hood for at least 5 minutes before turning hood on.
4. Add 10 milliliters of digestion acid mixture to each tube.
5. Place tubes to in the heating block.
6. Raise temperature to 180 °C watching carefully whether the samples start bumping or bubbling.
7. Gradually increase temperature to 210 °C heating tubes until white fumes are refluxing in the tube to ensure all samples are fully digested.
8. Cool to room temperature.
Complexing and Extraction

1. Add 1 milliliter concentrated hydrochloric acid and swirl.

2. Heat tubes at 150 °C for 30 minutes to reduce Se (VI) to Se (IV).

3. Cool to room temperature.

4. Dispense 15 milliliters of 0.1 M EDTA solution, 2 milliliters of DAN solution, and 5 milliliters of cyclohexane subsequently. *It is important to add DAN promptly after EDTA addition to avoid chelation of selenium by EDTA.

5. Vortex for 60 seconds.

6. Incubate in hot water bath at 60 °C for 40 minutes.

7. Turn on Fluorometer on to warm up for 30 minutes.

8. Remove from water bath and vortex for 1 minute.

9. Allow to settle and extract cyclohexane layer into Fluorometer cuvettes.

Fluorometry

1. Instrument
   Turner® Fluorometer Model 112

2. Lamp
   Turner® UV Lamp #110850

3. Operation condition
   a. Excitation: 369 nanometers (filter 7-60)
   b. Emission: 525 nanometers (filter 58) + Neutral Density Fiber (10%)
APPENDIX M, FLIER FOR RECRUITMENT OF SUBJECTS
ADVERTISEMENT FOR SUBJECTS

RESEARCH STUDY NEEDS VOLUNTEERS

Volunteers will be Paid $50.00 for Participation

Dr. Lydia Medeiros is doing a research study to learn how diabetic women and non-diabetic women use fat as they age. This study will investigate some factors that may explain why diabetes increases the risk for heart disease. Dr. Medeiros is a Faculty member in the Department of Human Nutrition and Food Management at The Ohio State University.

Women between ages 18-45 and 55-80 are needed as participants. All participants should be non-smokers, non-vegetarians and not hypertensive (with or without medication).

If you are interested in helping us learn more about the risk for heart disease in older diabetic women contact:

Dr. Lydia Medeiros  
Dept. of Human Nutrition and Food Management  
1787 Neil Avenue  
Columbus, OH 43210-1295  
(614) 292-2699
APPENDIX N, CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE FOR PRE- AND POST-MENOPAUSAL FEMALES
CONSENT TO INVESTIGATIONAL TREATMENT OR PROCEDURE

I, ________________________________, hereby authorize or direct Dr. Lydia Medeiros and associated of her choosing, to perform the following treatment or procedure (describe in general terms).

You are part of a study in which two groups of women (aged 18-45 or aged 55-80) will be compared. You should be a non-smokers, non-vegetarian and normotenive with or without medication. One group at each age is normal weight and non-diabetic, and the other group at each age is diabetic and perhaps overweight or obese. You should not be taking aspirin, aspirin-based or anti-inflammatory steroid drugs. If you are a young woman, you should not be pregnant or using oral contraceptives. Older women should be past menopause and not taking estrogen. As part of this study I will be asked to do these things:

1. I will complete a diet history and 3-day dietary record to learn HISTORICAL and USUAL daily food intake.
2. My height (without shoes), weight (light, in-door clothing) and my blood pressure will be measured.
3. I will provide a medical history to learn about chronic medical conditions and medications, including nutrient supplements, that could effect the study.
4. I will provide two samples of urine to test how my kidneys are working.
5. I will have a blood sample drawn from a vein in my arm. The total amount of blood drawn at each of two visits will be 21 cc or approximately 1 oz. (about 5 teaspoons).
6. I will make three visits to the metabolic laboratory of the Dept. of Human Nutrition and Food Management on the 3rd floor of Campbell Hall on the OSU campus. The building is handicapped accessed.

The experimental (research) portion of the treatment or procedure is:

Diabetes can lead to heart disease in later life. Young and older women with or without diabetes will be compared to learn the influence of gender and aging on fat production and function in the blood. serum and platelet fatty acids, serum triglycerides, cholesterol, and lipoprotein cholesterol, insulin and estrogen hormones, and platelet aggregation will be measured to study the effects of aging and diabetes on these measurements, and to learn more about the reason why diabetic women have different risk for heart disease.
This is done as part of an investigation entitled:

Insulin and Estrogen Control of Essential Fatty Acid Desaturation?

1. **Purpose of the procedure of treatment:**

   Information learned from this study will help us learn why diabetes can increase the risk for heart disease.

2. **Possible appropriate alternative procedures or treatment (not to participate in the study is always an option):**

   I may choose not to participate in this study.

3. **Discomforts and risks reasonably to be expected:**

   There should be no risk or discomforts caused by the collection diet history, medical history, height, weight, urine sample or blood pressure. There could be minimal risk and discomfort caused by the collection of the blood sample. Bruising and/or fainting could occur. A trained, experienced medical technologist will draw the blood sample. The risks and discomforts of blood drawing include pain from the needle, light-headedness, fainting, bruising, and rarely, infection. Sterile, disposable blood collecting supplies will be used. I may feel light-headed because you will be asking me not to eat or drink anything more than water after 9:00 pm the night before blood collection. Breakfast will be provided as soon as blood has been collected. I will be asked to not take aspirin, aspirin-based or anti-inflammatory steroid drugs two weeks prior to blood collection because these drugs interfere with the blood chemical tests being done as part of this study.

4. **Possible benefits for subjects/society:**

   I will receive $50.00 for participation at the completion of all data collection from me. Also, by participating in this study, I will have the satisfaction of knowing that the nutritional needs of aging women will be better understood.

5. **Anticipated duration of subject's participation (including number of visits):**

   I will be asked to come to the metabolic laboratory for the Endocrinology Clinic on the 5th floor of McCampbell Hall three times. This visit can occur at any time during the day since blood will not be collected and I will not be fasting. Instructions will be given on the collection of a three-day dietary intake record that I will complete at home on the days the investigator assigns to me. This will require approximately 5 minutes of recording after each meal for three days. I will provide a small urine sample so that we can be sure there are no problems with my kidneys. A medical conditions history, height, weight, and blood pressure...
will be recorded during this visit. The first visit will require approximately one hour to complete the various forms. On the second visit, I will be fasting and breakfast will be served after blood collection. Another urine sample will be collected. This will require approximately one hour, including the time spent eating breakfast. Blood will be collected again and breakfast served on the third visit. No other data will be collected so the visit will require approximately 30 minutes.

I hereby acknowledge that Dr. Lydia Medeiros has provided information about the procedure described above, about my rights as a subject, and she answered all questions to my satisfaction. I understand that I may contact her at Phone No. 292-2699 should I have additional questions. She has explained the risks described above and I understand them; she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that records obtained during my participation in this study that may contain my name or other personal identifiers may be made available to the sponsor of this study. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given to me concerning this treatment or procedure.

I understand in signing this form that, beyond giving consent, I am not waiving any legal right that I might otherwise have, and I am not releasing the investigator, the sponsor, the institution, or its agents form any legal liability for damages that they might otherwise have.

In the event of injury resulting from participation in this study, I also understand that immediate medical treatment is available at University Hospitals of the Ohio State University and that the costs of such treatment will be at my expense; financial compensation beyond that required by law is not available. Questions about this should be directed to the Office of Research Risks at 292-5958.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date: _______________ Time _________ pm Signed ______________________________ (subject)

Witness (es) _________________________ ______________________________
If Required _________________________ (Person Authorized to Consent for Subject if Required)
I certify that I have personally completed all blanks in this form and explained them to the subject for his/her representative before requesting the subject of his/her representative to sign it.

Date: ________________________ Signed: ______________________________________

(Signature of Project Director or her Authorized Representative)
APPENDIX O, QUESTIONNAIRES FOR MEDICAL CONDITIONS
INSULIN AND ESTROGEN CONTROL OF ESSENTIAL FATTY ACID DESATURATION
MEDICAL CONDITIONS QUESTIONNAIRE

Office Use Code #

Do you consider your residence: Rural _____ Suburban _____ Urban _____
Marital status ________

Circle the highest grade or year of regular school you have attended

Never attended school: or attended kindergarten only ________
Elementary 1 2 3 4 5 6 7 8
High school 9 10 11 12
College 1 2 3 4 5+
Did you complete the year marked school above? Yes _____ No _____

If you are post-menopausal:
did you experience surgical menopause? Yes ____ No ____ If yes, at what age ____
did you experience natural menopause? Yes ____ No ____ If yes, at what age ____

Have you had any recent weight changes? Yes ____ No ____
If yes, please describe __________________________

Does your work or daily activity primary involve the following?
______ sitting
______ standing
______ walking or other active exercise
______ heavy labor (such as heavy lifting, etc.)

Outside of your normal work or daily responsibilities, how often do you engage in exercise of 20 minutes or more which markedly increases your breathing (such as vigorous walking, cycling, running, swimming)
______ seldom or never
______ less than one time per week
______ one or two times per week
______ three to five times per week
______ six or more times per week

Are you on any type of special diet? Yes ____ No ____
If yes, please specify: __________________________

How long have you been on this diet? __________________

Food Allergies, or foods disliked or avoided? __________________
Other factors interfering with eating of digestion of food:  Yes _____  No _____
If yes, please specify: ___________________________________

Check-off the following health conditions that you have continued to experience in the past years or had diagnosed by a health practitioner or physician in the last year?

<table>
<thead>
<tr>
<th>Condition</th>
<th>In Past (before '93)</th>
<th>Present ('93 or '94)</th>
<th>Comments:</th>
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<tbody>
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<td>Anemia</td>
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<td>Asthma</td>
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<td>Hay fever</td>
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<td>Other allergy</td>
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<td>Malignancy</td>
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<td>Thyroid goiter</td>
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<td>Diabetes (w/ insulin)</td>
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<td>Age at onset</td>
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<td>Diabetes (wo insulin)</td>
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<td>Age at onset</td>
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<td>Hernia</td>
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<td>Hiatal hernia</td>
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<td>Kidney stones</td>
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<td>Urinary tract infections</td>
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<td>Rheumatic fever</td>
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<td>Stroke</td>
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<td>Arthritis</td>
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List other disease conditions ____________________________________________

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Please list any drug of medications you are currently taking:

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<tr>
<th>Name of Drug</th>
<th>Taken how often</th>
<th>Amount taken</th>
<th>Why prescribed</th>
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List additional drugs on back of page:

Do you have vitamin/ mineral and/or nutritional supplement? Yes ___ No ___
If yes, how frequently? Regularly ____ Occasionally ____
Taken within the last week? Yes ____ No ____

Please indicate the extent to which you personally have taken any of the following combination and/or single supplements within the last week. If you are not taking any particular brand supplement, leave the space blank

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<tr>
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<th>Brand/type:</th>
<th># Months taken</th>
<th>Dosage/day:</th>
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<td>Multivitamins</td>
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<td>Multivitamins w/iron</td>
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<td>Geritol</td>
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<td>Multiple vitamins w/ Minerals</td>
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<td>B complex</td>
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<td>fish oil pills</td>
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<td>rose hips</td>
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<td>Vitamin E</td>
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<td>Dolomite</td>
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<td>Bone meal</td>
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<td>Oyster shell</td>
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<td>Iron</td>
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<td>Evening primrose oil</td>
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<td>Others not listed</td>
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Weight: ___________ (lb) ___________ (kg) Height: ___________ (ft. in) ___________ (cm)

BMI (wt/hr²): ______________

Fasting glucose: ______________

Blood Pressure:

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<tr>
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<th>1 st visit:</th>
<th>2 nd visit:</th>
<th>3 rd visit:</th>
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<tr>
<td>Systolic</td>
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<td>Diastolic</td>
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<td>Pulse pressure</td>
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For pre-menopausal subject only:
Date of first day of your last menstrual period: ________________________________

Date of first blood collection: ________________________________ Time: ________________

Date of second blood collection ________________________________ Time: ________________

DO NOT EAT OR DRINK ANYTHING AFTER 9:00 PM THE NIGHT BEFORE BLOOD COLLECTION VISITS
APPENDIX P, THREE DAY DIET RECORDS
Instructions: Please write down all of the items you ate or drank either yesterday or for the last 3 days. Please include the way the food was prepared. Any sauces, gravies or other condiments that were eaten should be listed as a separate food item. Estimate the quantity of food consumed. Be as accurate as possible. If the food is an unusual food that we may not be familiar with please describe in the comment line.

<table>
<thead>
<tr>
<th>Food Item</th>
<th>How Prepared</th>
<th>Amount Eaten</th>
<th>Comment</th>
<th>Food Code</th>
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APPENDIX Q, 17-β ESTRADIOL DOUBLE ANTIBODY RADIOIMMUNOASSAY (RIA)
(Ref.: ICN RIA kit insert instruction)
17 β-ESTRADIOL DOUBLE ANTIBODY
RADIOIMMUNOASSAY (RIA)

Procedure

1. Label tubes as follows:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Sample</th>
</tr>
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<tbody>
<tr>
<td>1,2</td>
<td>Total count (TC)</td>
</tr>
<tr>
<td>3,4</td>
<td>Non-specific binding (NSB)</td>
</tr>
<tr>
<td>5,6</td>
<td>Total binding (0 pg/mL)</td>
</tr>
<tr>
<td>7,8</td>
<td>Standard A (10 pg/mL)</td>
</tr>
<tr>
<td>9,10</td>
<td>Standard B (30 pg/mL)</td>
</tr>
<tr>
<td>11,12</td>
<td>Standard C (100 pg/mL)</td>
</tr>
<tr>
<td>13,14</td>
<td>Standard D (300 pg/mL)</td>
</tr>
<tr>
<td>15,16</td>
<td>Standard E (1000 pg/mL)</td>
</tr>
<tr>
<td>17,18</td>
<td>Standard F (3000 pg/mL)</td>
</tr>
<tr>
<td>19,20</td>
<td>Unknown (samples)</td>
</tr>
</tbody>
</table>

2. Pipet 500 microliters of diluent buffer into tube 3 and 4 (NSB).

3. Pipet 50 microliters 0 pg/milliliter into tube 3, 4, 5 and 6.

4. Pipet 50 microliters of Standard A to F and samples in each tube.

5. Pipet 500 microliters of Estradiol $^{125}\text{I}$ Tracer into each tube.

6. Pipet 500 microliters of Anti-Estradiol into all tubes except total count (tube 1 and 2) and NSB (tube 3 and 4) tubes.

7. Vortex all tubes thoroughly.

8. Incubate for 90 minutes for 90 minutes.

9. Add 1 milliliter of Precipitant solution to each tube.

10. Vortex tubes thoroughly.
11. Centrifuge at 1500 x g (2840 rpm) at 2-8 °C for 20 minutes.  
   (If the pellet is not completely formed, centrifuge for another 10  
   minutes).

12. Aspirate the supernatant of each tube (except TC) into a radioactive  
    waste container.

13. Count radioactivity in each tube for one minute.

**Calculation**

Concentration of 17 β-estradiol:

\[
\text{a. } \% \frac{B}{B_0} = \frac{\text{CPM (sample)} - \text{CPM (NSB)}}{\text{CPM (0 pg/mL)} - \text{CPM (NSB)}} \times 100
\]

- CPM = Average counts of a duplicate
- Sample = Particular serum or standard being calculated
- NSB = Non-specific binding tube (blank)
- 0 pg/mL = Total binding tube (B₀ tube)

b. Construct a standard curve on a semi-log paper:
   - X-axis = log of concentrations of 17 β-estradiol
     Standards (10, 30, 100, 300, 1000, 3000 pg/mL)
   - y-axis = percent bound (\% \frac{B}{B_0})

c. Determine the 17 β-estradiol concentrations (pg/mL) of each  
   sample based on the standard curve.